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## CERTIFICATE

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I hereby certify the Complete Specification filed on 20 November 2003 with an application for Letters Patent number 529657 was made by Apotex Inc.

I further certify that annexed is a true copy of the Complete Specification as at the date of issue of this certificate in connection with Letters Patent 529657 in the name of Apotex Inc.

Dated 19 January 2005

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Neville Harris  
Commissioner of Patents, Trade Marks and Designs



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**CYCLOALKYL DERIVATIVES OF 3-HYDROXY-4-PYRIDINONES****FIELD OF THE INVENTION**

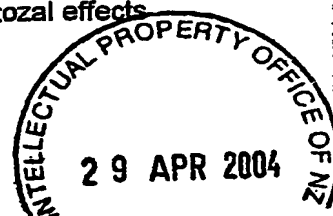
The invention relates to novel 3-hydroxy-4-pyridinone derivatives and their use in chelating ferric (Fe(III)) ions. More particularly, the invention relates to  
10 cycloalkyl derivative of 3-hydroxy-4-pyridinone.

**BACKGROUND**

3-Hydroxy-4-pyridinones are bidentate ligands that chelate to Fe(III) ion in the ratio of 3 : 1 and are useful in the removal of excess body iron in humans. Iron  
15 overload may be due to excess dietary consumption of iron, inherited genetic conditions such as haemochromatosis and regular blood transfusion. Regular blood transfusion is used to treat medical conditions such as thalassaemia, sickle cell anaemia, idiopathic haemochromatosis and aplastic anaemia. Increased iron absorption from transfusion leads to iron overload. Upon saturation of ferritin and  
20 transferrin in the body, iron deposit in many tissues such as the myocardium, liver and endocrine organs resulting in toxic effects.

The scope of iron chelator research and the proposed utility of chelators have been reviewed (Current Medicinal Chemistry, 2003, 10, 983-985, Tim F. Tam, et al). Iron chelators may be useful to prevent hydroxy radical formation, treatment of  
25 cancer, malaria, post-ischaemic reperfusion, and neurodegenerative diseases. Iron chelators such as Desferal<sup>TM</sup> (desferrioxamine mesylate) and Ferriprox<sup>TM</sup> (deferiprone) are used to remove excess body iron in thalassemia major patients because the human body has no effective means to excrete the iron accumulated from blood transfusion. Desferrioxamine is administered daily by subcutaneous  
30 infusion over a period of 8 to 12 hours. At present, deferiprone (1,2-dimethyl-3-hydroxy-4-pyridinone) is the only orally drug available. It undergoes extensive metabolism in the liver and more than 85% of the administered dose is recovered in the urine as the non-chelating O-glucuronide (Drug Metab. Dispo. 1992, 20(2), 256-261, S. Singh, et al.). A relatively high oral dose of 75 mg/kg (3.5 to 4 gm per day) is  
35 required for the treatment of iron overload conditions. Therefore, there is a need to identify a new orally active hydroxypyridinone with improved pharmacological properties than deferiprone.

Voest et. al. (Annals of Internal Medicine 1994, 120, 490-499) reviewed the clinical experience of iron chelators in non-iron overloaded conditions. Iron chelators  
40 were used to produce antioxidant effects, antiproliferative effects, antiprotozoal effects



5 and for aluminum chelation, and may be used be for a variety of disease state such  
as the treatment of rheumatoid arthritis, the protection against anthracycline cardiac  
poisoning, for limiting myocardial ischemia-reperfusion injury, as antitumour agents,  
and for the treatment of malaria. In addition, van Asbeck B. S. et. al. (J Clin Virol.  
2001 Feb;20(3):141-7) reported that iron chelators have anti-HIV activities. Therefore  
10 the utilities of iron chelators are not only restricted to the treatment of iron-overloaded  
conditions.

The members of the 3-hydroxy-4-pyridinones class are known for their ability  
to chelate iron. Prior art includes RE35,948, US6,448,273, US6,335,353 and  
US5,480,894. In US6,335,353, the ester prodrug derivatives of 3-hydroxy-4-  
15 pyridinones are used to facilitate efficient iron extraction from the liver, but none of  
the designed compounds has reached evaluation in humans.

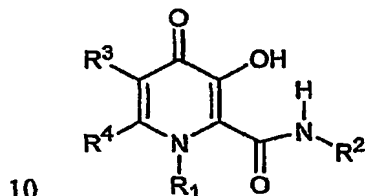
In other approaches, selected new compounds were designed to block the  
phase II metabolism of O-glucuronidation at the C3 oxygen of the deferiprone  
skeleton. US5,688,815 reported 1-alkyl-3-hydroxy-4-pyridinones with a C2 methyl  
20 group substituted with a phenyl or heteroyl ring and a hydroxy group, and the N1  
substituent is a lower alkyl. US6,335,353 described 1-alkyl-3-hydroxy-4-pyridinone  
with a C2 alkylcarbamoyl, or arylcarbamoyl, or aralkylcarbamoyl group and the N1-  
substituent is an aliphatic hydrocarbon group. The use of C2-methylcarbamoyl  
functionality in compound such as CP502 (1,6-Dimethyl-3-hydroxy-4(1H)-pyridinone-  
25 2-carboxy-(N-methyl)-amide hydrochloride; US 6,335,353) effectively blocked the O-  
glucuronidation at the C3 oxygen. Other analogues in US6,335,353 include CP506  
(1,6-Dimethyl-3-hydroxy-4(1H)-pyridinone-2-carboxy-(N-isopropyl)-amide  
hydrochloride), the C2-isopropylcarbamoyl analogue and CP508 (1,6-Dimethyl-3-  
hydroxy-4(1H)-pyridinone-2-carboxy-(N,N-dimethyl)-amide hydrochloride), the  
30 dimethylcarbamoyl analogue. CP502, CP506 and CP508 are prior art, and these  
compounds have not been evaluated in humans.



5

**SUMMARY OF THE INVENTION**

A first aspect of the present invention provides a novel 3-hydroxypyridin-4-one compound of formula I and its pharmaceutical acceptable salt:



wherein:

R<sup>1</sup> is X with the proviso that R<sup>2</sup> is Y;

or

15 R<sup>1</sup> is T with the proviso that R<sup>2</sup> is W;

X is C<sub>3</sub>-C<sub>6</sub> cycloalkyl;

Y is selected from the group C<sub>3</sub>-C<sub>6</sub> cycloalkyl; C<sub>1</sub> to C<sub>6</sub> alkyl;

T is selected from the group C<sub>1</sub> to C<sub>6</sub> alkyl;

20 W is C<sub>3</sub>-C<sub>6</sub> cycloalkyl;

R<sup>3</sup> is selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl;

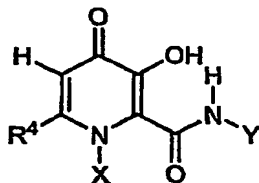
R<sup>4</sup> is selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl;

and its pharmaceutically acceptable salt thereof.

25 A second aspect of the present invention provides the use of the compounds of formula I in the treatment of iron overload related disease.

A third aspect of the invention provides pharmaceutical compositions comprising compound of formula I.

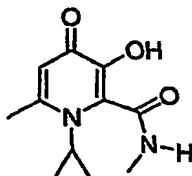
30 One preferred class of compounds of this invention is the compounds of formula I wherein R<sup>1</sup> is X with the proviso that R<sup>2</sup> is Y. X is C<sub>3</sub>-C<sub>6</sub> cycloalkyl; Y is C<sub>1</sub> to C<sub>6</sub> alkyl; R<sup>3</sup> is hydrogen; R<sup>4</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl.



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A still more preferred compound under this subset is a compound of formula I wherein  $R^4$  is methyl, X is cyclopropyl and Y is methyl, and the compound is 1-cyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid methylamide.

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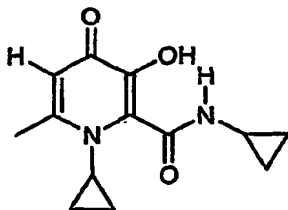


A second preferred class of compounds of this invention is the compounds of formula I wherein  $R^1$  is X with the proviso that  $R^2$  is Y. X is  $C_3$ - $C_6$  cycloalkyl; Y is  $C_3$ - $C_6$  cycloalkyl;  $R^3$  is hydrogen.  $R^4$  is  $C_1$ - $C_6$  alkyl.

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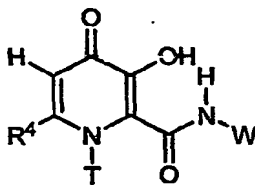
A preferred compound within this subset is a compound wherein  $R^4$  is methyl, X = Y = cyclopropyl, and the compound is 1-cyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclopropylamide.

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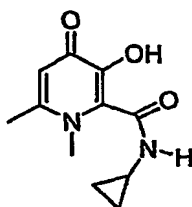
A third preferred class of compounds of compounds of formula I wherein  $R^1$  is T with the proviso that  $R^2$  is W; T is  $C_1$  to  $C_6$  alkyl; W is  $C_3$ - $C_6$  cycloalkyl.  $R^3$  is hydrogen.  $R^4$  is  $C_1$ - $C_6$  alkyl.

25



A more preferred compound under this subset is a compound wherein  $R^4$  is methyl, T is methyl and W is cyclopropyl, the compound is 3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclopropylamide.





In light of the above the present invention provides a group of cycloalkyl derivatives of 3-hydroxy-4-pyridinones having improved properties as compared to compounds reported in the prior art. The cycloalkyl group is attached to the N1 and/or C2 amido N atom. Prior to this application, compounds with N1-cycloalkyl substituent or C2 amido N-cycloalkyl substituent are unknown in the literature. These compounds are not prodrugs and have excellent metal ion selectivity. They show no complexation with essential metals such as calcium and magnesium at pH 7.4 in chemical assays. The  $D_{7.4}$  value is within the range of an established drug deferiprone and the compound is orally active in the iron overload rat model. These compounds are designed with favorable phenolic C3 OH pKas in the range of 8.3 to 8.8, a  $pFe^{3+}$  value of above 20, a smooth 1 : 3 ferric chelate formation as evident by Job's plot, and a  $D_{7.4}$  value  $> 0.1$ . The single crystal structure of the Fe(III) chelate confirms that the compound of formula I is a bidentate ligand.

#### **BRIEF REFERENCE TO THE DRAWINGS**

- FIG. 1 is a diagrammatic representation of Job's plot of Apo6622 (1-cyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclopropylamide), a compound of formula I.
- FIG. 2 is a diagrammatic representation of Job's plot of Apo6617 (1,6-dimethyl-3-hydroxy-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclopropylamide), a compound of formula I.
- FIG. 3 is a diagrammatic representation of Job's plot of Apo6619 (1-cyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid methylamide), a compound of formula I.
- FIG. 4 is a speciation plot for  $Fe^{3+}$ -Apo6619.
- FIG. 5 is a speciation plot for  $Fe^{3+}$ -Apo6617.
- FIG. 6: Effectiveness of Apo6619 and Apo6617 in Promoting Urinary Iron Excretion in the Iron Overloaded Rat ( $n=6$ ).
- FIG. 7: Single crystal structure of  $Fe(Apo6617)_3$  chelate.



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TABLE 1: Chemical properties of compound of formula I.

TABLE 2: Metal binding selectivity of Apo6619.

TABLE 3: Effectiveness of Apo6619 and Apo6617 in Promoting Fecal Iron Excretion in the Iron Overloaded Rat (n=6). Values are expressed as  $\mu\text{g/day/kg}$ .

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TABLE 4: Effectiveness of Apo6619 and Apo6617 in Promoting Urinary and Fecal Iron Excretion in the Iron Overloaded Rats (n=6/group). Values are expressed as  $\mu\text{g/day/kg}$ . Fecal excretion values 3 days after chelator administration are given and compared to the baseline values determined 3 days prior to chelator administration. Values are expressed as mean  $\pm$  1SD.

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TABLE 5: Crystal data and structure refinement for  $\text{Fe}(\text{Apo6617})_3$ .

TABLE 6: Bond lengths [ $\text{\AA}$ ] and angles [ $^\circ$ ] for  $\text{Fe}(\text{Apo6617})_3$ .

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#### **DETAILED DESCRIPTION OF THE INVENTION**

As used herein:

Alkyl means a branched or unbranched saturated hydrocarbon chain having, unless otherwise noted, one to six carbon atoms, including but not limited to methyl, ethyl, propyl, isopropyl, n-propyl, butyl, sec-butyl, isobutyl, n-pentyl, hexyl.

25

The term "cycloalkyl" as employed herein alone or as part of another group includes saturated cyclic hydrocarbon groups containing 1 ring, including monocyclic alkyl, containing a total of 3 to 6 carbons forming the ring, which includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl.

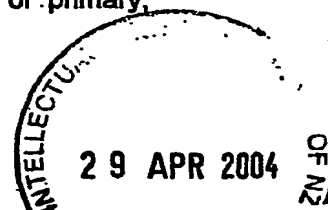
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Pharmaceutically acceptable, non-toxic salts refer to pharmaceutically acceptable salts of the compounds of this invention, which retains the biological activity of the parent compounds and are not biologically or otherwise undesirable (e.g. the salts are stable). Salts of the two types may be formed from the compounds of this invention: (1) Salts of inorganic and organic bases from compounds of formula I, which has a phenol functional group, and (2) Acid addition salts may be formed at the amine functional group of compounds of formula I of this invention.

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Pharmaceutically acceptable salts derived from inorganic bases include sodium, potassium, lithium, ammonium, calcium, and magnesium salts. Particularly preferred are the sodium, calcium and magnesium salts. Pharmaceutically acceptable, non-toxic salts derived from organic bases include salts of primary,

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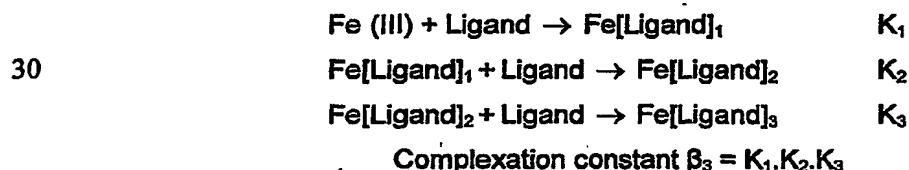
5 secondary and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins. Such salts are exemplified by, for example, 2-amino-2-hydroxymethyl propane 1,3-diol, isopropopylamine, tromethamine, glucosamine, methylglucamine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like.

10 Pharmaceutically acceptable acid addition salts are formed with inorganic and organic acids such as halo acids, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, and ethanesulfonic acid.

The term "animals" refers to humans as well as all other animal species, particularly mammals (e.g. dogs, cats, horses, cattle, pigs, etc.), reptiles, fish, insects  
15 and helminths.

Compounds of this invention are designed to show improve properties to known deferiprone analogues. One criteria used in the design of oral active chelators in the 3-hydroxy-4-pyridinone series are compounds having  $pFe^{3+}$  value higher than deferiprone ( $pFe^{3+} = 19.7$ ). The definition of  $pFe^{3+}$  used herein is the  
20 concentration of ferric ion in solution when the total amount of iron equals  $10^{-6}$  M and the concentration of ligand is  $10^{-5}$  M and pH is 7.4. It is calculated using experimental determined  $pK_a$  and metal complexation constant using Hyperquad software (Version 2.1, Peter Gans, University of Leeds). The lowering of the  $pK_a$  value of the C3 phenolic OH to less than 8.8 ensures that a higher  $pFe^{3+}$  value when  
25 combined with a favorable complexation constant  $\beta_3$ . The concept of complexation is detailed below.

The stepwise and overall complexation constants of a bidentate ligand such as 3-hydroxy-4-pyridinone is shown below:



The iron chelator drug deferiprone (1,2-dimethyl-3-hydroxy-4-pyridinone) chelates iron with a complexation constant ( $\log \beta_3$ ) of 36.3 and a  $pFe^{3+}$  of 19.7. The  
35  $pK_a$ s of deferiprone is 3.56 and 9.64. Most compounds of this invention have similar complexation constant ( $\log \beta_3$ ) in the range of 34 to 36, a  $pFe^{3+} > 20$  and favorable phenol  $pK_a$  values of 8.3 to 8.8. Accordingly, compounds of this invention are excellent chelators of Fe(III). The theoretically calculated human jejunum effective permeability [ $P_{eff}$ ] of compounds of this invention is predicted by computational  
40 calculations using QMPRPlus<sup>TM</sup> software (from Simulationplus inc.). Most





5 compounds of this invention have calculated  $P_{eff}$  in the range of  $1 \pm 0.3$  (cm/s  $\times 10^{-4}$ ), implying that the compounds have good human jejunal permeability. The chemical properties of representative compounds of formula I are shown in Table 1.

10 Compounds of formula I with cycloalkyl groups at  $R^1$  and/or  $R^2$  are metal chelators with high  $pFe^{3+}$  values. The  $D_{7.4}$  values of compounds of formula I are similar to deferiprone and further study in iron overload rats showed that compounds of formula I are effective in the removal of iron *in vivo*. The details of the animal efficacy study are shown in the examples below.

15 Compounds of formula I do not bind essential metals such as manganese, calcium and magnesium. The  $pM$  values and complexation constants of a representative compound of formula I are shown in Table 2 (and discussed in more detail in example 11). The compound has preference for the binding  $Fe^{3+}$  than other bivalent and trivalent metals such as Cu, Zn and Al.

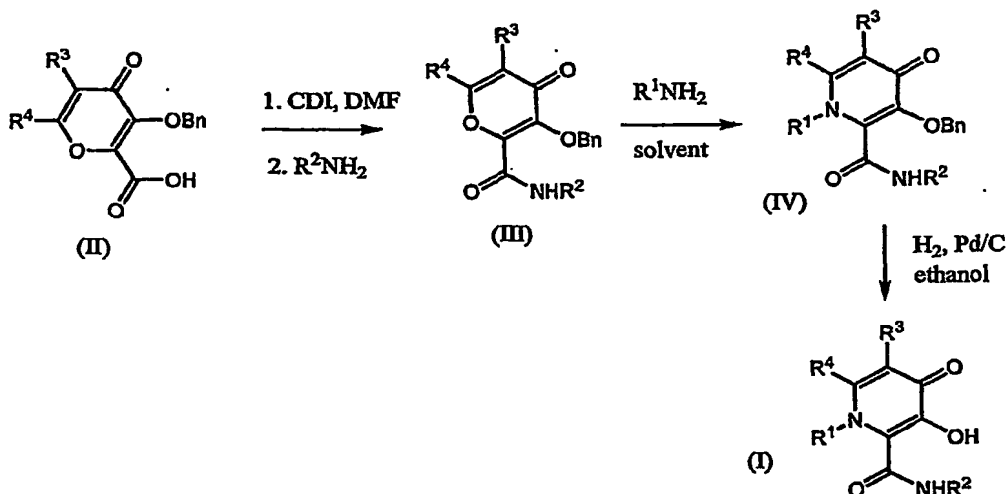
20 Compounds of formula I are novel cycloalkyl derivatives of 3-hydroxy-4-pyridinones. It has  $pFe^{3+}$  value above 20, a favorable  $D_{7.4}$  value comparable to deferiprone, a preference towards the chelation of  $Fe^{3+}$  and a C2-alkylcarbamoyl or C2-cycloalkylcarbamoyl moiety that is designed to block the phase II metabolism of the 3-OH group.

25 In addition to the above, compounds of formula I binds  $Fe^{3+}$  in the ratio of 1 : 3 at physiological conditions at pH 7.4. The Job's plot analysis confirms the 1 : 3 ratio of chelator to ferric metal (Fig. (1 to 3) and example 9).

30 The speciation plots of the Fe-complex Vs. different pHs can be calculated by using Hyperquad Stimulation and Speciation software (HYSS2 © 2000 Protonic Software) with the input of experimental  $pKas$  (example 7 and 8) and the complexation constants  $K_1$ ,  $K_2$  and  $K_3$  (example 11). Figures 4 and 5 illustrate the speciation plot of compounds of formula I at different pHs. In both studies, representative compounds of formula I exclusively form  $FeL_3$  chelate at pH above 7.0. Consequently, there should be no concern over the formation of  $FeL_2^+$  or  $FeL^{2+}$  at physiological pH. The absence of these species ensures no exposed iron is present *in-vivo* at the physiological pH of 7.4.

35 Compounds of formula I wherein  $R^1$  is X with the proviso that  $R^2$  is Y; or  $R^1$  is T with the proviso that  $R^2$  is W; is prepared according to the method outlined in Scheme 1.



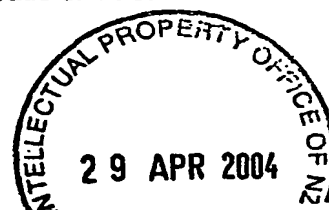


Scheme 1

Acid (II) is reacted with 1,1'-carbonyldiimidazole in an inert solvent for 2 to 5 hrs, preferably 5 hrs in an inert solvent at elevated temperature between 30 to 70°C, followed by the addition of an amine  $R^2NH_2$ , the compound (III) is isolated by conventional means. A solution of (III) and an amine  $R^1NH_2$  in an inert solvent such as an alcohol is heated at 50 to 80°C to effect the amine insertion of (III) for a period of 3 to 48 hrs, to effect the formation of compound (IV). Hydrogenation of compound (IV) in alcohol over a hydrogenation catalyst affords Compound (I), which is isolated by conventional means. The preferred hydrogenation catalyst is palladium on carbon or palladium hydroxide on carbon and Raney Ni. The preparation of the starting material acid (II) is reported in US6,426,418.

Compounds of formula I is tested in iron overloaded rats. The fecal iron excretion and urinary excretion data for representative compounds Apo6617 and Apo6619 are shown in Tables 3 and 4, and Fig 6, respectively. Both compounds showed significant fecal iron excretion when compared to control at an oral dose of 113 and 450  $\mu\text{mol/kg}$ . Further, Apo6619 and Apo6617 facilitate the urinary excretion of iron significantly higher than deferiprone at 450  $\mu\text{mol/kg}$ . Both compounds are considered more potent than deferiprone in iron mobilization in iron overloaded rats.

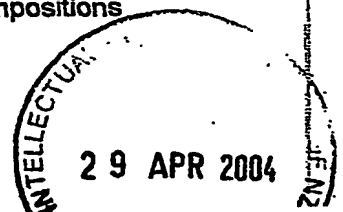
The 3-dimensional single crystal structure of  $\text{Fe}(\text{Apo6617})_3$  has been determined and is shown in Figure 7. The preparation of  $\text{Fe}(\text{Apo6617})_3$  and the crystal structure parameters can be found in Example 13 below. This proves that compounds of formula I are bidentate ligands and chelates  $\text{Fe}(\text{III})$  in the ratio of 1 : 3.



5 For the treatment of iron overloaded diseases such as thalassemia, sickle cell  
disease and haemochromatosis, and the treatment of patients having a toxic  
concentration of iron, the compounds of the invention may be administered orally,  
topically, or parenterally in dosage unit formulations containing conventional non-  
toxic pharmaceutically acceptable carriers, adjuvants and vehicles. For the treatment  
10 of non-iron overloaded conditions such as HIV infection, protective effect against  
anthracycline cardiac poisoning, cancer and malaria, the compounds of this invention  
may also be administered orally, topically, or parenterally in dosage unit formulations  
containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants  
and vehicles. The term parenteral as used herein includes subcutaneous injection or  
15 infusion techniques. In addition to the treatment of warm-blooded animals such as  
mice, rats, horses, cattle, sheep, dogs, cats, etc., the compounds of the invention are  
effective in the treatment of humans.

For compositions, conventional non-toxic solid carriers include, for example,  
pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium  
20 saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like  
may be used. The active compound as defined above may be formulated as liquid  
pharmaceutically administrable compositions can, for example, be prepared by  
dissolving, dispersing, etc. an active compound as defined above and optional  
pharmaceutically adjuvants in a carrier, such as, for example, water, saline, aqueous  
25 dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If  
desired, the pharmaceutical composition to be administered may also contain a  
minor amount of non-toxic auxiliary substances such as wetting or emulsifying agents  
and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine  
sodium acetate, triethanol-amine oleate, etc. Actual methods of preparing such  
30 dosage forms are known, or will be apparent to those skilled in this art: for example,  
see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa.,  
15th Edition, 1975, ch. 83 p.1436-1460, and ch. 89 p.1576-1607. The composition of  
formulation to be administered will, in any event, contain a quantity of the active  
compound(s) in an amount effective to alleviate the symptoms of the subject being  
35 treated.

The pharmaceutical compositions containing the active ingredient may be in a  
form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily  
suspensions, dispersible powders or granules, emulsions, hard and soft capsules, or  
syrups or elixirs. Compositions intended for oral use may be prepared according to  
40 any method known to the art for the manufacture of pharmaceutical compositions



5 and such compositions contain one or more agents from the group consisting of  
sweetening agents, flavoring agents, coloring agents and preserving agents in order  
to provide pharmaceutically elegant and palatable preparations. Tablets contain the  
active ingredient in admixture with the non-toxic pharmaceutically acceptable  
excipients, which are suitable for the manufacture of tablets. The excipients may be  
10 for example, inert diluents, such as calcium phosphate or sodium phosphate;  
granulating and disintegrating agents, for example, corn starch, or alginic acid;  
binding agents, for example starch, gelatin or acacia; and lubricating agents, for  
example magnesium stearate, stearic acid or talc. The tablets may be coated by  
known techniques to delay the disintegration and absorption in the gastrointestinal  
15 tract and thereby provide a sustained action over a long period. For emollient,  
emulsifier, or moisturer, monostearate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules  
wherein the active ingredients are mixed with an inert solid diluent, for example,  
calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient  
20 is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive  
oil.

Aqueous suspensions contain the active materials in admixture with the  
excipient suitable for the manufacture of aqueous suspensions. Such excipients are  
suspending agents, for example sodium carboxymethylcellulose, methylcellulose,  
25 hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum and gum  
acacia; dispersing or wetting agents may be a naturally-occurring phosphate, for  
example lecithin, or condensation products of an alkene oxide with fatty acids, for  
example polyoxyethylene stearate, or condensation products of ethylene oxide with  
long chain aliphatic alcohols, for example heptadecathyl-eneoxycetanol, or  
30 condensation products of ethylene oxide with partial esters derived from fatty acids  
and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous  
suspensions may also contain one or more preservatives, for example ethyl, or n-  
propyl, p-hydroxybenzoate, one or more coloring agents, such as sucrose or  
saccharin.

35 Oily suspensions may be formulated by suspending the active ingredient in a  
vegetable oil, for example rachis oil, olive oil, sesame oil or coconut oil, or in a  
mineral oil such as liquid paraffin. The oily suspensions may contain a thickening  
agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such  
as those set forth above, and flavoring agents may be added to provide a palatable



- 5 oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with the dispersing or wetting agent, suspending agent and one or more preservatives.

10 Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional recipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical composition of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these.

15 Suitable emulsifying agents may be naturally occurring phosphates, esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents.

20

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, orbital or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be formulated according to the known art using those suitable

25 dispersing or wetting agents and suspending agents, which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solutions and isotonic sodium chloride solution. In

30 addition, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation or injectables.

Compounds of formula (I), or if appropriate a pharmaceutically-acceptable salt thereof and/or a pharmaceutically-acceptable solvate thereof, may also be

35 administered as a topical formulation in combination with conventional topical excipients. Examples of topical formulations are ointments, creams or lotions, impregnated dressings, gels, gel sticks, spray and aerosols. The formulations may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams. They may also contain

40



5 compatible conventional carriers, such as cream or ointment bases and ethanol or  
oleyl alcohol for lotions. Topical formulations are envisaged where appropriate, to  
contain an amount of actives to alleviate the symptoms of the subject being treated.  
Suitably, the compound of formula (I), or if appropriate a pharmaceutically-acceptable  
salt thereof, will comprise from about 0.5 to 10% by weight of the formulation.  
10 Suitable cream, lotion, gel, stick, ointment, spray or aerosol formulations that may be  
used for compounds of formula (I) or if appropriate a pharmaceutically-acceptable  
salt thereof, are conventional formulations well known in the art, for example, as  
described in standard text books Remington's Pharmaceutical Sciences, Mack  
Publishing Company, Easton, Pa., 15th Edition, 1975, ch. 83 p.1436-1460, and ch.  
15 89 p.1576-1607.

Parenteral administration is generally characterized by injection, either  
subcutaneously, intramuscularly or intravenously. Injectables can be prepared in  
conventional forms, either as liquid solutions or suspension in liquid prior to injection,  
or as emulsions. Suitable excipients are for example, water, saline, dextrose,  
20 glycerol, ethanol or the like. In addition, if desired, the pharmaceutical compositions  
to be administered may also contain minor amounts of non-toxic auxiliary substance  
such as wetting or emulsifying agents, pH buffering agents and the like, such as for  
example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc.

The amount of active ingredient that may be combined with the carrier  
25 materials to produce a single dosage form will vary depending upon the host treated,  
and the particular mode of administration of humans may contain from 0.5 mg to 5  
gm of active agent compounded with an appropriate and convenient amount of carrier  
material which may vary from about 5 to about 95% of the total composition. Dosage  
unit forms will generally contain between from about 1 mg to about 500 mg of an  
30 active ingredient.

It will be understood, however, that the specific dose level for any particular  
patient will depend upon a variety of factors including the activity of the specific  
compound employed, the age, body weight, general health, sex, diet, time of  
administration, drug combination and the severity of the particular disease  
35 undergoing therapy.

The compounds of the present invention differ from those compounds  
reported in US6,448,273, US6,335,353, RE35,948 and US5,688,815. The first three  
patents describe 3-hydroxy-4-pyridinones having a N1 aliphatic hydrocarbon group.  
US5,688,815 also describes 3-hydroxy-4-pyridinones with a N1 substituted or  
40 unsubstituted lower alkyl group. According to a standard chemistry textbook,



5 Organic Chemistry by James B. Hendrickson, Donald J. Cram, George S. Hammond,  
third edition, 1970, McGraw Hill, p.72, aliphatic hydrocarbons are composed of  
chains of carbon atoms not arranged in rings. Substances belonging to this group  
are sometimes referred as open chain compounds. Examples of aliphatic  
hydrocarbon group are linear or branched alkyls such as methyl, ethyl, propyl,  
10 isopropyl, isobutyl, butyl and tert-butyl. The compounds of this invention consist of 3-  
hydroxy-4-pyridinones with (a) N1-cycloalkyl substituent and C2 cycloalkylcarbamoyl  
substituent; or (b) N1-cycloalkyl substituent and C2 cycloalkylcarbamoyl substituent;  
or (c) N1-alkyl substituent with C2 cycloalkyl-carbamoyl substituent. They are  
compounds with acyclic hydrocarbon substituents. In acyclic hydrocarbons, the  
15 carbon chains form rings. Examples of acyclic hydrocarbon groups are cycloalkyl  
derivatives such as cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. The four  
patent US6,448,273, US6,335,353, RE35,948 and US5,688,815, do not cover  
cycloalkyl derivatives of 3-hydroxy-4-pyridinones. This invention covers 3-hydroxy-4-  
pyridinones with a N1-cycloalkyl group with an alkylcarbamoyl group at C2 or a  
20 cycloalkylcarbamoyl group at C2. It also covers 3-hydroxy-4-pyridinones with a  
cycloalkylcarbamoyl group at C2 with a N1-alkyl group.

The invention is further described and illustrated in the following specific  
examples.

25

### **SPECIFIC DESCRIPTION OF THE MOST PREFERRED EMBODIMENTS**

#### **Example 1:**

Preparation of 3-Benzyloxy-6-methyl-4-oxo-4H-pyran-2-carboxylic acid  
cyclohexylamide.

30

1,1'-carbonyldiimidazole (1.99 g, 12.30 mmol) was added to a solution of the  
3-(benzyloxy)-6-methyl-4-oxo-4H-pyran-2-carboxylic acid (2.0 g, 7.69 mmol) in DMF  
(10 ml) at room temperature. The resulting solution was heated at 40°-50°C for 3 hrs.  
A light yellow solution was observed. Cyclohexylamine (1.23 ml, 10.76 mmol) was  
35 then added. The resulting mixture was stirred at room temperature for overnight.  
The DMF was removed under reduced pressure to give light yellow oil as a crude  
product, which was purified by flash column chromatography (elution gradient: from  
1 : 1 ethyl acetate/ hexane to 10% methanol in ethyl acetate) to yield the titled  
40 compound (1.60 g, yield 61%) as white solid.

40



- 5 M.p. 118-120 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.91 (m, 2H, cyclohexyl-H), 1.29 (m, 2H, cyclohexyl-H), 1.58 (m, 3H, cyclohexyl-H), 1.79 (m, 2H, cyclohexyl-H), 2.37(s, 3H, CH<sub>3</sub>), 3.79 (m, 1H, CH), 5.40(s, 2H, CH<sub>2</sub>), 6.28(s, 1H, CH), 7.41(m, 5H, ArH), 7.67 (br, 1H, NH); MS (m/z) 342 (M<sup>+</sup> +1).

- 10 In a similar manner, by replacing cyclohexylamine with other amine, the following compounds are prepared:

3-Benzyloxy-6-methyl-4-oxo-4H-pyran-2-carboxylic acid cyclopropylamide.

- 15 M.p. 79-80 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.21 (m, 2H, cyclopropyl-H), 0.70 (m, 2H, cyclopropyl-H), 2.35 (s, 3H, CH<sub>3</sub>), 2.71 (m, 1H, CH), 5.35 (s, 2H, CH<sub>2</sub>), 6.27 (s, 1H, CH), 7.39 (m, 5H, ArH), 7.70 (s, 1H, NH); <sup>13</sup>C(CDCl<sub>3</sub>) δ 6.35, 7.21, 19.87, 22.61, 22.70, 75.56, 115.37, 128.94(2C), 129.17(2C), 129.25, 135.49, 146.14, 146.39, 160.22, 165.74, 176.17; MS (m/z) 300 (M<sup>+</sup> +1).

- 20 3-Benzyloxy-6-methyl-4-oxo-4H-pyran-2-carboxylic acid methylamide.

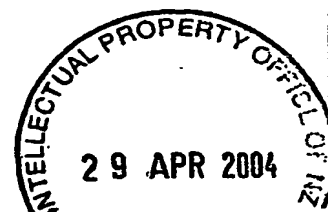
M.p. 137-140 °C, <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 2.38 (s, 3H, CH<sub>3</sub>), 2.78 (d, 3H, NCH<sub>3</sub>), 5.39 (s, 2H, CH<sub>2</sub>), 6.30 (s, 1H, CH), 7.40 (m, 5H, ArH), 7.62 (br, 1H, NH); MS (m/z) 300 (M<sup>+</sup> +1).

25

#### Example 2:

Preparation of 3-Benzyloxy-1,6-dimethyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclohexylamide.

- 30 To a solution of 3-benzyloxy-6-methyl-4-oxo-4H-pyran-2-carboxylic acid cyclohexylamide (1.40 g, 4.1mmol) in 5ml of methanol, methylamine solution (9 ml of 2M solution in methanol, 16 mmol) was added. The resulting solution was stirred at 70 to 75°C for overnight under the pressure in a sealed tube. The solvent was removed under reduced pressure gave light yellow solid as a crude product. The material was purified by column chromatography (elution gradient: 100% ethyl acetate to 25% methanol in ethyl acetate) to give the titled compound as white solid (1.20g, 83.0%). M.p. 258-260°C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 1.26-1.45 (m, 6H, cyclohexyl-H), 1.79 (m, 2H, cyclohexyl-H), 1.95(m, 2H, cyclohexyl-H), 2.41(s, 3H, CH<sub>3</sub>), 3.82( s, 3H, NCH<sub>3</sub>), 3.95 (m, 1H, CH), 5.13 (s, 2H, CH<sub>2</sub>), 7.19 (s, 1H, CH), 7.36 (m, 3H, ArH), 7.43(m, 2H, ArH), 8.50 (br, 1H, NH); MS (m/z) 355 (M<sup>+</sup> +1).
- 40





5 In a similar manner, by replacing 3-benzyloxy-1,6-dimethyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclohexylamide with 3-benzyloxy-1,6-dimethyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclopropylamide, 3-benzyloxy-1,6-dimethyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclopropylamide was prepared.

10 M.p. 187-189°C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.52 (m, 2H, cyclopropyl-H), 0.74 (m, 2H, cyclopropyl-H), 2.18 (s, 3H, CH<sub>3</sub>), 2.78 (m, 1H, CH), 3.50 (s, 3H, NCH<sub>3</sub>), 5.08 (s, 2H, CH<sub>2</sub>), 6.12 (s, 1H, CH), 7.33 (m, 3H, ArH), 7.39(m, 2H,ArH), 7.91 (br, 1H, NH); MS (m/z) 313 (M<sup>+</sup> +1).

15 3-Benzyloxy-1-cyclopropyl-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid methylamide.

M.p. 132-135°C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 1.05 (m, 4H, cyclopropyl-H), 2.38 (s, 3H, CH<sub>3</sub>), 2.70 (d, J=1.8Hz, 3H, NCH<sub>3</sub>), 3.35 (m, 1H, CH), 5.07 (s, 2H, CH<sub>2</sub>), 6.14(s, 20 1H, CH), 7.15 (br., 1H), 7.35 (m, 5H, ArH); <sup>13</sup>C(CDCl<sub>3</sub>) δ 9.48, 20.30, 25.86, 34.15, 74.01, 118.16, 127.79, 128.06(2C), 128.22(2C), 137.35, 142.05, 143.98, 149.91, 162.01, 173.89; MS (m/z) 313 (M<sup>+</sup> +1).

In a similar manner, the following compounds are prepared:-

25 3-Benzyloxy-1-cyclopropyl-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclopropylamide.

M.p. 164-167°C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.54 (m, 2H, cyclopropyl-H), 0.76 (m, 2H, cyclopropyl-H), 1.08-1.11 (m, 4H, cyclopropyl-H), 2.35 (s, 3H, CH<sub>3</sub>), 2.75 (m, 1H, 30 CH), 3.37 (m, 1H, CH), 5.05 (s, 2H, CH<sub>2</sub>), 6.13 (s, 1H, CH), 7.33 (m, 5H, ArH), 7.89 (br, s, 1H, NH); MS (m/z) 339 (M<sup>+</sup> +1).

### Example 3:

35 3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclohexylamide [Apo6621].

Pd(OH)<sub>2</sub> on charcoal (0.18 g, 10% w dry basis) was added to a solution of 3-benzyloxy-6-methyl-4-oxo-4H-pyran-2-carboxylic acid cyclohexylamide (1.0 g, 2.82 mmol) in ethanol (50 ml) under nitrogen. The mixture was hydrogenated at 50 psi for 4 hrs. The Pd(OH)<sub>2</sub> was removed by filtration through a layer of the Celite, and the 40 Celite cake was washed with ethanol (3x10 ml). The ethanol filtrate was evaporated to give an off-white solid (0.57 g, 77%). Further purification by recrystallization from



5 methanol (15 ml) gave the title compound as a white solid (0.18 g). M.p. 280-285 °C (dec); <sup>1</sup>H-NMR (CD<sub>3</sub>OD 400 MHz) δ 1.30-1.43 (m, 5H, cyclohexyl-H), 1.70 (m, 1H, cyclohexyl-H), 1.80 (m, 2H, cyclohexyl-H), 2.00 (m, 2H, cyclohexyl-H), 2.41 (s, 3H, CH<sub>3</sub>), 3.63 (s, 3H, CH<sub>3</sub>), 3.90 (m, 1H, CH), 6.38 (s, 1H, CH); MS (m/z) 265 (M<sup>+</sup> +1).

10 In a similar manner, by replacing 3-benzyloxy-6-methyl-4-oxo-4H-pyran-2-carboxylic acid cyclohexyl amide with other 2-carboxylic acid cycloalkyl amide, the following compounds are prepared: 3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclopropylamide [Apo6617].

15 M.p. 260-262°C; <sup>1</sup>H-NMR (MeOD-*d*<sub>4</sub>, 400 MHz) δ 0.66 (m, 2H, cyclopropyl-H), 0.85 (m, 2H, cyclopropyl-H), 2.41 (s, 3H, CH<sub>3</sub>), 2.95 (m, 1H, CH), 3.63 (m, 1H, NCH<sub>3</sub>), 6.38(s, 1H, CH); MS (m/z) 223 (M<sup>+</sup> +1).

20 1-Cyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid methylamide [Apo6619]

M.p. 258-260°C (dec); <sup>1</sup>H-NMR (MeOD-*d*<sub>4</sub>, 400 MHz) δ 1.05 (m, 2H, cyclopropyl-H), 1.19 (m, 2H, cyclopropyl-H), 2.54 (s, 3H, CH<sub>3</sub>), 2.97 (s, 1H, NCH<sub>3</sub>), 3.46 (m, 1H, CH), 6.33 (s, 1H, CH); MS (m/z) 223 (M<sup>+</sup> +1).

25

#### Example 4:

Preparation of 3-(benzyloxy)-*N*-cyclobutyl-6-methyl-4-oxo-4H-pyran-2-carboxamide.

A mixture of 3-(benzyloxy)-6-methyl-4-oxo-4H-pyran-2-carboxylic acid (2.5 g, 9.6 mmol, 1.0 equiv), N, N'-carbonyldiimidazole (2.49 g, 15.37 mmol, 1.6 equiv) in 30 DMF (20 mL) was stirred at 50°C for 5 h. The mixture was cooled to room temperature. Cyclobutylamine hydrochloride (1.24 g, 11.52 mmol, 1.2 equiv) and Et<sub>3</sub>N (1.74 mL, 12.48 mmol, 1.3 equiv) was added, and the mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure. Purification by chromatography (1 : 1 hexanes/EtOAc, then EtOAc) provided the titled 35 compound (2.76 g, 91.56%) as a yellow solid. M.p. 69.3-71.0°C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 1.51-1.72 (m, 4H, cyclobutyl H), 2.19-2.28 (m, 2H, cyclobutyl H), 2.37 (s, 3H, CH<sub>3</sub>), 4.39-4.41 (m, 1H, CH), 5.41 (s, 2H, OCH<sub>2</sub>Ph), 6.30 (s, 1H, CH), 7.39-7.49 (m, 5H, ArH), 7.86 (br, 1H, NH), and MS (m/z) 314 (M<sup>+</sup> +1), 217, 91.

40

Proceeding in a similar manner, the following compounds are prepared:  
3-(benzyloxy)-*N*-cyclopentyl-6-methyl-4-oxo-4H-pyran-2-carboxamide



5 M.p. 108.0-108.5 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 1.11-1.16 (m, 2H, cyclopentyl H), 1.50-1.55 (m, 4H, cyclopentyl H), 1.87-1.92 (m, 2H, cyclopentyl H), 2.38 (s, 3H, CH<sub>3</sub>), 4.17-4.22 (m, 1H, CH), 5.41 (s, 2H, CH<sub>2</sub>), 6.30 (s, 1H, CH), 7.38-7.43 (m, 5H, ArH), 7.72 (br, 1H, NH), MS (m/z) 328(M<sup>+</sup> +1), 217, 91.

10 **Example 5:**

Preparation of 3-(benzyloxy)-*N*-cyclobutyl-1,6-dimethyl-4-oxo-1,4-dihydropyridine-2-carboxamide.

To a solution of the compound from example 4 (2.616 g, 8.35 mmol, 1.0 equiv) in methanol (10 ml) was quickly added Methylamine (2M in Methanol, 20 ml, 40 mmol, 4.79 equiv). The sealed tube was stirred overnight at 70-75°C. The resulting brown solution was evaporated to dryness and purified by chromatography (EtOAc, then 1:4 MeOH/EtOAc) provided the titled compound (1.70 g, 62.24%) as a white solid. M.p. 221.3-222.4°C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 1.65-1.69 (m, 2H, cyclobutyl H), 1.90-1.95 (m, 2H, cyclobutyl H), 2.14-2.21 (m, 2H, cyclobutyl H), 2.31 (s, 3H, CH<sub>3</sub>), 3.42 (s, 3H, NCH<sub>3</sub>), 4.34-4.30 (m, 1H, CH), 5.05 (s, 2H, OCH<sub>2</sub>Ph), 6.22 (s, 1H, CH), 7.39-7.30 (m, 5H, ArH), 9.08-9.06 (d, 1H, J=7.08 Hz, NH); MS (m/z) 327(M<sup>+</sup> +1), 230, 166, 91.

Proceeding in a similar manner, the following compounds are prepared:

3-(benzyloxy)-*N*-cyclopentyl-1,6-dimethyl-4-oxo-1,4-dihydropyridine-2-carboxamide

25 M.p. 233.6-234.4°C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 1.43-1.52 (m, 4H, cyclopentyl H), 1.54-1.60 (m, 2H, cyclopentyl H), 1.78-1.83 (m, 2H, cyclopentyl H), 2.30 (s, 3H, CH<sub>3</sub>), 3.43 (s, 3H, NCH<sub>3</sub>), 4.13-4.18 (m, 1H, CH), 5.04 (s, 2H, OCH<sub>2</sub>Ph), 6.22 (s, 1H, CH), 7.30-7.41 (m, 5H, ArH), 8.80-8.82 (d, J=6.95 Hz, 1H, NH); MS (m/z) 341(M<sup>+</sup> +1), 230, 166, 91.

30

**Example 6:**

Preparation of *N*-cyclobutyl-3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridine-2-carboxamide [Apo 6222].

A mixture of *N*-cyclobutyl-3-benzyloxy-1,6-dimethyl-4-oxo-1,4-dihydropyridine-2-carboxamide (1.528 g, 4.68 mmol, 1.0 equiv), 10% Pd on activated carbon (200 mg, wet), and ethanol (200 ml) was stirred under 50 psi of H<sub>2</sub> at room temperature for 2.5 h. The catalyst was filtered through Celite and the filtrate was evaporated to give a solid, which was recrystallized from MeOH gave the titled compound (0.57 g, 51.5%) as a white solid. M.p. 277.3°C (dec); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 1.68-1.70 (m, 2H, cyclobutyl H), 1.95-2.01 (m, 2H, cyclobutyl H), 2.20-2.26 (m, 2H,

40



- 5 cyclobutyl H), 2.29 (s, 3H, CH<sub>3</sub>), 3.41 (s, 3H, NCH<sub>3</sub>), 4.31-4.35 (m, 1H, CH), 6.13 (s, 1H, CH), 8.98 (br, 1H, NH); MS (m/z) 237 (M<sup>+</sup> +1), 185, 166, 123.

Proceeding in a similar manner, the following compounds are prepared:  
*N*-cyclopentyl-3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydro-pyridine-2-carboxamide

- 10 [Apo 6620]

M.p. 289.3°C (dec); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 1.49-1.55 (m, 4H, cyclopentyl H), 1.61-1.68 (m, 2H, cyclopentyl H), 1.83 -1.87 (m, 2H, cyclopentyl H), 2.29 (s, 3H, CH<sub>3</sub>), 3.42 (s, 3H, NCH<sub>3</sub>), 4.14-4.18 (m, 1H, CH), 6.12 (s, 1H), 8.71-8.73 (d, J=7.05 Hz, 1H, NH); MS (m/z) 251 (M<sup>+</sup> +1), 166.

1-Cyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid  
 cyclopropylamide [Apo 6618].

- 20 M.p. 241-143°C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 0.53 (m, 2H, cyclopropyl-H), 0.71 (m, 2H, cyclopropyl-H), 0.94-1.00 (m, 4H, cyclopropyl-H), 2.42 (s, 3H, CH<sub>3</sub>), 2.79 (m, 1H, CH), 3.30 (m, 1H, CH), 6.08 (s, 1H, CH), 8.54 (br, s, 1H, NH); MS (m/z) 249 (M<sup>+</sup> +1).

- 25 **Example 7:**  
 pKa determination for Apo6619 by potentiometric titration.

- pKa values of ligands were determined by potentiometric titration when a ligand concentration greater than 1x10<sup>-2</sup> M in water could be prepared. In a typical experiment, the sample solution (2.67x10<sup>-2</sup> M) was prepared by the following method: Apo6619 (92.6 mg) was weighed into a 25-ml beaker, followed by the addition of 0.1 M NaCl (15 ml). The mixture was sonicated for 10 minutes to give a clear colorless solution. Nitrogen gas was then allowed to bubble through the solution. 1.000 N Hydrochloric acid (624 µl, 1.5 equivalent) was added to the solution to give pH 1.88.
- 35 The solution was allowed to equilibrate at 22°C for 60 minutes.

- The solution was then titrated against 1.000 N NaOH at 22°C to reach pH 11.8. For each addition of base, the solution was allowed to equilibrate until a constant pH reading was reached. The volume of the base added and the pH reading were recorded for each measurement. 137 measurements were taken to
- 40 finish the experiment.



5        The data set of pH vs. base volume was analyzed using Hyperquad 2000 (Version 2.1, Peter Gans, University of Leeds). Given the model:  $L^- + H^+ \leftrightarrow LH$  ( $pK_{a1}$ ) and  $LH + H^+ \leftrightarrow LH_2^+$  ( $pK_{a2}$ ), the  $pK_a$  values of Apo6619 were optimized as  $pK_{a1} = 8.6$  and  $pK_{a2} = 2.5$ .

10

**Example 8:**

**pKa determination for Apo6617 by spectrophotometric titration:**

15         $pK_a$  values of ligands can be determined by spectrophotometric titration when both the conjugated acid and base absorb in the UV-Visible region. In a typical experiment, the sample solution was prepared by the following method: Apo6617 (0.792 mg) was weighed into an 80-ml beaker, followed by the addition of 0.1 M NaCl (50 ml). The mixture was sonicated for 5 minutes to give a clear colorless solution. Nitrogen gas was allowed to bubble through the solution. 1.000 N NaOH (50  $\mu$ l) was  
20        added to give pH 10.9. The solution was allowed to equilibrate at 22°C for 1 hour. A sipper system was used for the circulation of the sample solution between the beaker and the flow cell.

25        The sample solution was titrated against standard hydrochloric acid solutions at 22°C to reach pH 1.40. After each addition of acid the solution was allowed to equilibrate until a constant pH reading was reached. The pH and the UV-Vis spectrum were recorded for each measurement. The peak wavelengths of the deprotonated species ( $L^-$ ), the neutral species (LH), and the protonated species ( $LH_2^+$ ) were 314 nm, 281 nm, and 249 nm, respectively. In the region of pH > 6, after each addition of acid there was a slight decrease in the absorbance at 314 nm and a  
30        slight increase at 281 nm in each spectrum, whereas in the region of pH < 5, after each addition of acid there was a slight decrease in the absorbance at 281 nm and a slight increase at 249 nm in each spectrum. The solution was titrated until there was no obvious change in the spectra after several subsequent additions of acid. 116 measurements were taken to finish the experiment.

35        The resulting data set was then analyzed using pHAB (Peter Gans, University of Leeds). The  $pK_a$  values of Apo6617 were optimized as  $pK_{a1} = 8.6$  and  $pK_{a2} = 2.5$ .



**5 Example 9:**

Stoichiometry of Fe-Apo6622 complexes by Job's method:

In a typical experiment, Fe-Apo6622 complex solutions were prepared by mixing a stock solution of  $\text{Fe}^{3+}$  (atomic absorption standard, 1005  $\mu\text{g}/\text{ml}$  in 1 wt.% HCl, Aldrich) and a stock solution of Apo6622 ( $6.98 \times 10^{-3}$  M in 0.1 M MOPS pH 7.4). 12 sample solutions were prepared. While the sum of the total iron concentration ( $C_{\text{total}}^{\text{iron}}$ ) and the total ligand concentration ( $C_{\text{total}}^{\text{L}}$ ) in each of the 12 sample solutions was kept constant ( $8.00 \times 10^{-4}$  M), the molar fraction of the ligand,  $\alpha$  ( $\alpha = C_{\text{total}}^{\text{L}} / (C_{\text{total}}^{\text{L}} + C_{\text{total}}^{\text{iron}})$ ), for the 12 sample solutions were different and were prepared as 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.75, 0.8, 0.9, and 1.0, respectively. The total volume for each of the 12 sample solutions was 5 ml, using MOPS (0.1 M, pH 7.4) as the solvent. The pH of the 12 solutions was adjusted by adding NaOH to pH 7.4. The sample solutions were vortexed at room temperature for 3 hours, and then placed in a Dubnoff Metabolic Shaking Incubator at 25°C and at 90 RPM overnight. The sample solutions were centrifuged at 4000 rpm for 15 minutes, and then placed back in the incubator at 25°C without shaking. The UV-Vis spectrum was recorded at 25°C for each of the 12 solutions.

A Job's plot was created with the absorbance at 450 nm as the y-axis and  $\alpha$  as the x-axis. A maximum absorbance was found at  $\alpha = 0.75$ , which corresponds to an iron : ligand ratio of 1:3 in the complexes. The Job's plot result is shown in Fig. (1).

Proceeding in a similar manner, the Job's plots of Fe-Apo6617 and Fe-Apo6619 were created. They are shown in Fig. (2) and (3).

**30 Example 10:**

Distribution coefficient determination:

MOPS buffer (50 mM, pH=7.4) and 1-octanol were used as the aqueous phase and the organic phase, respectively, for distribution coefficient determinations. The MOPS buffer and 1-octanol were pre-saturated with each other before use.

In a typical experiment, an organic stock solution of Apo6618 (1-cyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclopropylamide) was prepared by weighing out 0.50 mg of the compound into a 10-mL volumetric flask and bringing to volume with 1-octanol. The solution was then sonicated for 60 minutes so that the sample could dissolve completely. The concentration of the stock solution was calculated as  $C_{\text{org}}^0 = 2.0 \times 10^{-4}$  M. The organic standard solution of



5 Apo6618 with  $2.0 \times 10^{-5}$  M was prepared in a 10-mL volumetric flask by 10  
times dilution of the stock solution with 1-octanol. The sample solution was prepared  
in a 10-mL volumetric flask. The stock sample solution (3 ml) was pipetted into the  
flask followed by the addition of MOPS buffer (3 ml). The standard and sample  
10 solutions were then vortexed for 2 hours. After vortexing, the solutions were  
transferred to test tubes and centrifuged at 4000 rpm for 15 minutes. UV-Vis spectra  
were recorded for the standard solution and the organic (top) phase of the sample  
solution at 22°C. The distribution coefficient,  $D_{7.4}$ , was calculated using the following  
equation:

$$15 \quad D_{7.4} = [A_{\text{org}} / (C_{\text{org}}^0 \epsilon_{\text{org}} - A_{\text{org}})] \times (V_{\text{aqu}} / V_{\text{org}})$$

Where  $\epsilon_{\text{org}}$  = the molar extinction coefficient of the peak wavelength ( $\lambda_{\text{max}}$ )  
obtained from the UV-Vis spectrum of the organic standard solution;  $A_{\text{org}}$  =  
absorbance of the organic phase in the sample solution at the same  $\lambda_{\text{max}}$ ;  $C_{\text{org}}^0$  = the  
20 concentration of the stock solution;  $V_{\text{aqu}}$  = the volume of MOPS buffer in the sample  
solution;  $V_{\text{org}}$  = the volume of the stock solution in the sample solution.

#### Example 11:

Determination of metal complexation constants.

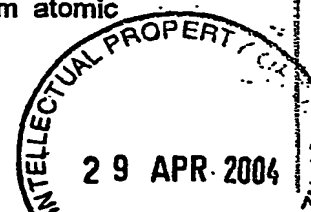
25

#### A. Instrumental and chemicals:

A pH meter (Accumet Research AR15, 13-636-AR15, Fisher) and a  
combination electrode (Accumet Standard-size Glass Combination Electrode, 13-  
620-285, Fisher) were used for pH measurements. Before using, the electrode was  
30 calibrated with three standard buffer solutions (pH 4.00, pH 7.00, and pH 10.00,  
Fisher). The titrant was added manually by using digital pipettes (Eppendorf). An  
UV-visible spectrophotometer (Agilent 8453) was used for UV-Vis absorbance  
measurements.

A sipper system (89068D Agilent) was used whenever pH-dependent absorbencies  
35 were measured. A vortexer (VX-2500 Multi-tube Vortexer, VWR Scientific Products)  
was used for the preparation of sample solutions in both distribution coefficient and  
Job's plot experiments.

The metal stock solutions were purchased from Aldrich: Iron atomic  
absorption standard solution (1000 µg/ml of Fe in 1 wt. % HCl); Aluminum atomic  
40 absorption standard solution (1000 µg/ml of Al in 1 wt. % HCl); Calcium atomic



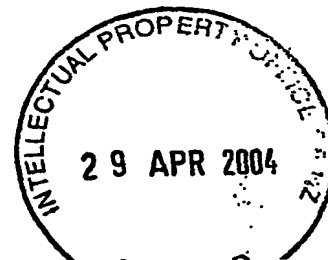
5 absorption standard solution (1000  $\mu\text{g/ml}$  of Ca in 1 wt. % HCl); Copper atomic  
absorption standard solution (1000  $\mu\text{g/ml}$  of Cu in 1 wt. %  $\text{HNO}_3$ ); Magnesium atomic  
absorption standard solution (1000  $\mu\text{g/ml}$  of Mg in 1 wt. %  $\text{HNO}_3$ ); Manganese atomic  
absorption standard solution (1000  $\mu\text{g/ml}$  of Mn in 1 wt. %  $\text{HNO}_3$ ); Zinc atomic  
absorption standard solution (1000  $\mu\text{g/ml}$  of Zn in 1 wt. % HCl). The standard Sodium  
10 Hydroxide and Hydrochloric acid solutions were purchased from VWR Scientific  
Products. MOPS (3-[N-Morpholino]propanesulfonic acid) was purchased from  
Sigma-Aldrich.

B. Determination of stepwise formation constants for Fe-Apo6619 system by  
15 spectrophotometric titration. Apo6619 is 1-cyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-  
dihydro-pyridine-2-carboxylic acid methylamide.

Stepwise formation constants for  $\text{M}^{n+}$ -ligand systems were determined by  
spectrophotometric titration when metal complexes have a strong absorbance in the  
20 visible region due to ligand to metal charge transfer. In a typical experiment, the  
sample solution was prepared according to the following method: APO6619 (10.7  
mg) was weighed into an 80-ml beaker, followed by the addition of 0.1 M NaCl (50  
ml). The mixture was sonicated for 10 minutes to give a clear colorless solution. Iron  
stock solution (atomic absorption standard, Aldrich, 496  $\mu\text{l}$ ,  $8.93\text{E-}06$  moles) was  
25 pipetted into the solution followed by the addition of 1.000 N NaOH (137  $\mu\text{l}$ ). The  
molar ratio between the total iron and the total APO6619 was 1:5.4. The mixture was  
allowed to equilibrate at room temperature overnight. Nitrogen was allowed to  
bubble through the solution. 1.000 N Hydrochloric acid (3 ml) was then added to the  
solution to give pH 1.5. The solution was allowed to equilibrate at  $22^\circ\text{C}$  for 3 hours.

30 A sipper system was used for the circulation of the sample solution between  
the beaker and the flow cell.

The sample solution was titrated against standard NaOH solutions at  $22^\circ\text{C}$  to  
reach pH 6.89. After each addition of base the solution was allowed to equilibrate  
until a constant pH reading was reached. The pH and the UV-Vis spectrum were  
35 recorded for each measurement. For each measurement enough base was added  
so that there was a slight increase in the absorbance of the spectrum. The solution  
was titrated until there was no obvious increase in the spectra after several  
subsequent additions of base. Altogether 64 measurements were taken to finish the  
experiment.





5 The resulting data set was then analyzed using pHAB. Given the model:  $L^- + H^+ \leftrightarrow LH$  ( $pK_{a1}$ ),  $LH + H^+ \leftrightarrow LH_2^+$  ( $pK_{a2}$ ),  $Fe^{3+} + L^- \leftrightarrow FeL^{2+}$  ( $K_1$ ),  $FeL^{2+} + L^- \leftrightarrow FeL_2^+$  ( $K_2$ ),  $FeL_2^+ + L^- \leftrightarrow FeL_3$  ( $K_3$ ), and  $\beta_3 = K_1K_2K_3$ , the stepwise formation constants for Fe-Apo6619 system were optimized as  $\log K_1 = 12.5(1)$ ;  $\log K_2 = 11.6(1)$ ;  $\log K_3 = 9.5(1)$ ;  $\log \beta_3 = 33.6(2)$ .

10

C. Determination of stepwise formation constants for Al-Apo6619 system by potentiometric titration.

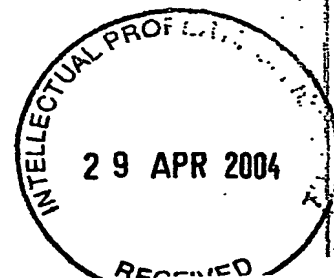
Stepwise formation constants for  $M^{n+}$ -ligand system were determined by potentiometric titration when metal complexes ( $\geq 0.002$  M) do not precipitate during titration. In a typical experiment, the sample solution was prepared by the following method: APO6619 (31.91 mg) was weighed into a 25-ml beaker followed by the addition of 0.1 M NaCl (18.9 ml). The mixture was sonicated for 10 minutes to give a clear colorless solution. Aluminum stock solution (atomic absorption standard, Aldrich, 971  $\mu$ l,  $3.59 \times 10^{-5}$  mole) was pipetted into the solution followed by the addition of 1.000 N NaOH (229  $\mu$ l) to give pH 8.56. The molar ratio between the total Aluminum and the total APO6619 was 1:4. For  $M^{2+}$  metals, a molar ratio of 1:3 was used. Nitrogen was allowed to bubble through the solution. The mixture was allowed to equilibrate at 22°C for 2 hours. 1.000 N Hydrochloric acid (264  $\mu$ l) was then added to the solution to give pH 2.20. The solution was allowed to equilibrate at 22°C for 1 hour.

The solution was titrated against 1.000 N NaOH at 22°C to reach pH 11.0. For each addition of base, the solution was allowed to equilibrate until a constant pH reading was reached. The volume of the base added and the pH reading were then recorded for each measurement. 93 measurements were taken to finish the experiment.

The data set of pH vs. base volume was analyzed using Hyperquad 2000. Given the model:  $L^- + H^+ \leftrightarrow LH$  ( $pK_{a1}$ ),  $LH + H^+ \leftrightarrow LH_2^+$  ( $pK_{a2}$ ),  $Al^{3+} + L^- \leftrightarrow AlL^{2+}$  ( $K_1$ ),  $AlL^{2+} + L^- \leftrightarrow AlL_2^+$  ( $K_2$ ),  $AlL_2^+ + L^- \leftrightarrow AlL_3$  ( $K_3$ ), and  $\beta_3 = K_1K_2K_3$ , the stepwise formation constants for Al-Apo6619 system were optimized as  $\log K_1 = 12.6(2)$ ;  $\log K_2 = 9.2(1)$ ;  $\log K_3 = 8.4(1)$ ;  $\log \beta_3 = 30.2(2)$ .

#### Calculation of $pM^{n+}$

$pM^{n+}$  is defined as  $-\log[M(H_2O)_m]^{n+}$  at physiological conditions, i.e.: pH 7.4, a ligand concentration of 10  $\mu$ M, and a metal concentration of 1  $\mu$ M.



- 5 To calculate  $pM^{n+}$  for a  $ML_n$  system,  $\beta_n$  and  $pK_a$  values are needed ( $\beta_n$  are the formation constants for  $M^{n+} + n L^- \leftrightarrow ML_n$ ;  $pK_a$  are the equilibrium constants for  $L^- + n H^+ \leftrightarrow LH_n^{(n-1)+}$ ). The  $pM^{n+}$  can be calculated by using Hyss software (Hyperquad Stimulation and Speciation software: HYSS2 © 2000 Protonic Software).

- 10 The data obtained from the above determinations for compounds of formula I can be found in Table 1 and 2.

**Example 12:**

Evaluation of compounds of formula I in iron overloaded rats.

15

Effectiveness of Apo6619 and Apo6617 in Promoting Urinary and Fecal Iron Excretion in the Iron Overloaded Rat.

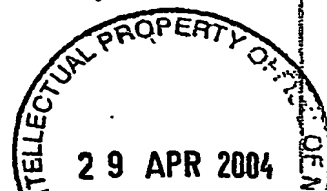
- The purpose of this study was to determine the effectiveness of Apo6619 and Apo6617 in promoting iron excretion in the iron overloaded rat model. Iron overloading was achieved by administration of iron dextran. Iron overloading using iron dextran has previously been used to assess chelator efficacy in mice (Kontoghiorghe G. J., *Mol Pharmacol.* 1986, 30(6), 670-3; Bartfay et al., *Cardiovasc Res.* 1999, 43(4), 892-900), gerbils (Hershko et al., *J. Lab Clin Med* 2002, 139, 50-58), rats (Rakba N. *Biochem Pharmacol.* 1998, 55(11):1796-1806) and primates (Bergeron et. al., *Blood*, 1992, 79(7), 1882-1890). The iron loading regime used in this study results in a 20-fold increase in liver iron and a 3.8-fold increase in cardiac iron levels in male rats. Previous studies in this model have demonstrated that this model is not associated with significant abnormalities in animal weight gain, food consumption, clinical chemistry or hematology parameters.

30

Experimental Protocol:

- Six male Sprague-Dawley rats (weighing between 200-250 gms) were received from Charles River Laboratories, Montreal, Quebec, Canada. Rats were iron loaded by administration of iron dextran intraperitoneally at a dose of 100 mg/kg, twice weekly for a period of 4 weeks for a total of 8 injections (iron dextran, Sigma). The total volume of iron dextran injected was 1 mL/kg. Following an eight week period, rats were transferred to metabolic cages (one rat/cage). Once the animals were in the metabolic cages, excreta (both urine and feces) were collected daily for at least 3 day prior to and 4 days following the administration of each chelator. Each of the two chelators (Apo6619 and Apo6617) was administered consecutively.

40



- 5 Chelators were administered as a single dose of 450  $\mu$ moles/kg by oral gavage at a dose volume of 2-4 mL/kg. The animals were weighed prior to dosing to enable exact dosage administration. Animals were checked daily (eyes, skin and movement) after chelator administration to determine if there were any obvious signs of ill health. Urine and feces were stored at -20°C until analysis for total iron concentrations.

#### Animal Diet, Water and Housing:

- 15 Rats were housed in a climate and light controlled environment (temperature: 19-25°C, relative humidity 40%, 12hrs light/dark cycle) throughout the study. During the acclimatization, iron loading and equilibration phases, rats were placed in standard cages (2 rats/cage), fed standard rodent chow and given regular tap water *ad libitum*. Rats were transferred to metabolic cages (one rat/cage, Nalgene, Rochester NY) after iron loading and equilibration. Three days before placement of the rats in the metabolic cages, rats were fed a low iron diet (3 ppm iron, Dyets Inc., Bethlehem, PA) and given Millipore water *ad libitum*. Rats were continued on the low iron diet for the duration of the study. The purpose of placing animals on a low iron diet was to reduce the background noise produced by dietary iron in the fecal samples.

#### Preparation of dosing solution of chelators:

- 25 A 50 mg/ml dosing solution of the chelator of formula I (570 mg) was first dissolved in a mixture of Millipore water (2 ml) and 6N HCl (0.4 ml) and brought up to the final volume (11.4 ml) with Millipore water (9 ml). Final pH of the solutions was adjusted to pH 4 with diluted sodium hydroxide solution. Solutions were protected from light and prepared freshly prior to each administration.

#### Iron Determinations:

- 35 ~~Urine and feces samples were shipped~~ to the Trace Elements Laboratory at the London Health Sciences Center, London, Ontario, Canada for analysis of total iron concentration. Briefly, feces samples were prepared by adding water, heating to 98°C, vortexing and subsequently freeze drying. The samples were then mixed, and a representative subsample taken and digested with boiling HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>. Feces samples were then diluted 1:100 with ultrapure water prior to running using a high resolution sector field ICP-MS (Finnigan Element 1). Urine samples were digested with 0.1% HNO<sub>3</sub> and diluted 1/10 prior to running on the ICP-MS. Appropriate

- 5 calibration curves using iron spiked samples and NIST traceable standards were used. Samples lying above or below the quantification range were re-run. Since the total amount of urine and feces produced over a given period of time was known as well as rat weight, total iron levels in the urine and feces are expressed as  $\mu\text{g/day/kg}$ . Statistical comparisons within and between groups was made using unpaired t-tests.
- 10 A p value of  $<0.05$  was accepted as significant.

### Results

- The rats showed no obvious signs of ill health following administration of any of the chelators. All animals continued to gain weight normally after each of the chelators was administered.
- 15

#### Urinary Excretion:

- The effectiveness of Apo6619 and Apo6617 in promoting urinary iron excretion is presented in Figure 6, below. Baseline urinary excretion, as measured during the 3 days prior to Apo6619 administration was  $6 \pm 1 \mu\text{g/day/kg}$ .
- 20

- This increased to  $240 \pm 131 \mu\text{g/day/kg}$  one day after Apo6619 administration ( $p=0.007$ ). Excretion subsequently declined to  $16 \pm 5 \mu\text{g/day/kg}$  the second day after Apo6619 administration, however even these levels were still significantly higher than baseline ( $p=0.004$ ). By the third day, urinary iron excretion had returned to baseline levels ( $5 \pm 1 \mu\text{g/day/kg}$ ). Apo6617 also resulted in increased iron excretion one and two days after administration ( $164 \pm 55$  and  $17 \pm 13 \mu\text{g/day/kg}$ , respectively). Although the urinary excretion produced by Apo6619 was numerically greater than that achieved with Apo6617 ( $240 \pm 131 \mu\text{g/day/kg}$  versus  $164 \pm 55 \mu\text{g/day/kg}$ , one day after chelator administration), this difference did not achieve statistical significance due to the fact that one of the six rats exhibited higher urinary excretion with Apo6617 than Apo6619.
- 25
- 30

- For comparative purposes, deferiprone was also studied at a dose of 450  $\mu\text{moles/kg}$  in the above model but in a different set of rats ( $n=6$ ). The baseline urinary iron levels measured  $9 \pm 3 \mu\text{g/day/kg}$ . These were increased to  $80 \pm 32 \mu\text{g/day/kg}$  one day after deferiprone administration ( $p=0.06$ ) and levels returned to baseline by the second day.
- 35



5 Fecal Excretion:

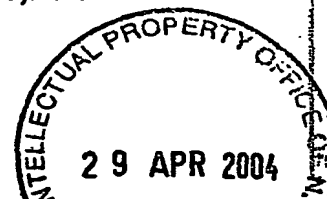
The effectiveness of Apo6619 and Apo6617 in promoting fecal iron excretion is presented in Table 3. Both the baseline values as well as the post-chelator induced values represent the sum of iron excreted in the three days prior to and following chelator administration, respectively. Both Apo6619 and Apo6617  
10 increased fecal iron excretion at 450  $\mu\text{moles/kg}$ , but this reached statistical significance only in the Apo6617 group (Apo6619:  $4154 \pm 1245 \mu\text{g/day/kg}$ ,  $p=0.08$  versus baseline; Apo6617  $4411 \pm 790 \mu\text{g/day/kg}$  versus baseline,  $p=0.008$ ). In a previous study in the same model, deferiprone administered to six rats at a dose of 450  $\mu\text{moles/kg}$  resulted in fecal iron excretion values of  $2157 \pm 169 \mu\text{g/day/kg}$  three  
15 days after chelator administration.

Second Rat Study at 113  $\mu\text{moles/kg}$ :

A second study was conducted to confirm the efficacy results of Apo6619 and Apo6617 observed in the above study and to further characterize the efficacy of  
20 these compounds at doses lower than 450  $\mu\text{mol/kg}$ . The study was conducted in two separate groups of iron overloaded rats. The method of iron overloading, preparation of dosing solutions and assessment of efficacy in these rats was similar to that described in the above study.

The first group of rats ( $n=6$ ) were treated with Apo6619 consecutively at  
25 doses of 28, 113 and 450  $\mu\text{moles/kg}$ . Similarly, the second group of rats (also  $n=6$ ) was treated with Apo6617 at these same three doses. A summary of the excretion data is shown in Table 4. Similar to the previous study, both Apo6619 and Apo6617 produced an increase in urinary iron excretion at the 450  $\mu\text{moles/kg}$  dose (Apo6619:  $11 \pm 3$  at baseline to  $335 \pm 76$  1-day post-Apo6619,  $p=0.0001$ ; Apo6617:  $14 \pm 4$  at  
30 baseline to  $183 \pm 20$  1-day post-Apo6617,  $p=0.0003$ ). In contrast to the previous study where no significant difference between the urinary efficacy of Apo6619 and Apo6617 was observed at 450  $\mu\text{moles/kg}$ , in this study it was clear that Apo6619 was more effective than Apo6617 ( $p=0.004$ ) at this dose. Similarly, at 113  $\mu\text{mol/kg}$  (25 mg/kg), both Apo6619 and Apo6617 increased urinary excretion ( $p<0.005$ ), but  
35 Apo6619 was more effective than Apo6617 ( $p=0.01$ ). At 28  $\mu\text{moles/kg}$ , only Apo6617 produced an increase in iron excretion ( $p=0.01$ ) above baseline. However, the magnitude of the increased excretion was small for both Apo6619 and Apo6617.

Fecal excretion was increased by Apo6619 at 450  $\mu\text{moles/kg}$  ( $p=0.03$ ) and there was a trend towards increased excretion with Apo6617 as well ( $p=0.08$ ). No



- 5 significant increases in fecal excretion were detectable with either chelator at doses lower than 450  $\mu$ moles/kg.

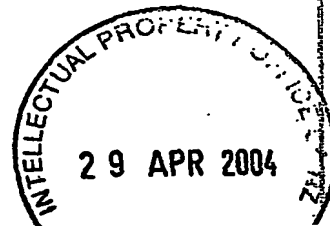
Collectively, both studies show that Apo6619 and Apo6617 result in increased urinary and fecal iron excretion. This excretion is superior to that observed in historical studies with deferiprone in the same model. While Apo6619 produces  
10 significantly greater urinary iron excretion as compared to Apo6617 at high doses, the superiority of Apo6619 over Apo6617 in producing increased fecal iron excretion was not evident from these studies. In large part, this is due to both the high, and highly variable "background" levels of iron in the feces (i.e. low signal to noise ratio) making chelator induced increases in iron excretion difficult to detect.

15

**Example 13:****Preparation of Fe(Apo6617)<sub>3</sub> chelate**

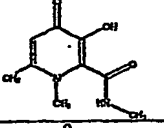
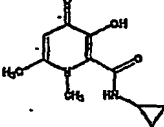
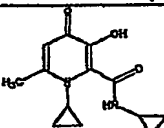
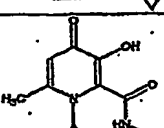
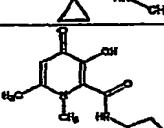
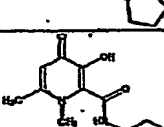
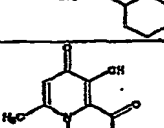
A pH 9.7 carbonate buffer was prepared by dissolving 0.84g of sodium  
20 bicarbonate, 1.06g of sodium carbonate in de-ionized water and diluting the solution to 50ml. Apo6617 (1.028g, 4.62 mmol) was added to the carbonate buffer (25ml). The heterogeneous mixture was stirred for 15 minutes at room temperature to give a clear solution. Anhydrous ferric chloride (0.2417g, 1.49 mmol) was added in small portions over 5 min. at room temperature to give a dark red solution. The flask was  
25 then sealed with a septum cap and stirred at room temperature for 42 h. Acetonitrile (30 ml) was added and the solvent was evaporated under reduced pressure to give a dry red mass. The solid was dissolved in dichloromethane (90 ml), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The crude product was subjected to purification by flash chromatography using elution gradient  
30 (dichloromethane/methanol mixture: 95/5, 90/10, 85/15 and 80/20). A red solid (900mg) was obtained. The solid was mixed with the mixture of ethyl acetate/methanol (90/10, 60 ml) and stirred at RT for 1 h. The insoluble particulate was filtered and the filtrate evaporate under reduce pressure to give the product (800.5 mg). MS (m/z): 742.6 (M<sup>+</sup> + Na), 720.4 (M<sup>+</sup> + 1), 634.6, 499.0, 469.6, 360.4,  
35 334.3

A saturated solution of the Fe(Apo6617)<sub>3</sub> was prepared by dissolving 0.2 gm of the material in dichloromethane. The insoluble particulate was filtered. 0.3 ml of ethyl acetate was added to 1 ml of the saturated dichloromethane solution in a vial. The vial was capped at room temperature. The dark brown crystals were removed for



5 crystallography determination. The 3-dimension single crystal structure is shown in Figure 7. The crystallographic data is shown in Table 5 to 6.

TABLE 1: Chemical Properties of compounds of formula I.

Structure	Compound #	D <sub>7.4</sub>	pK <sub>a</sub> s	Log β <sub>3</sub>	pFe <sup>8+</sup>	QMPR Plus™ software Cal. Human Jejunal Effective Permeability [cm/s x 10 <sup>-4</sup> ]
	CP502	0.04	2.7, 8.5	33.6	20.9	0.81
	Apo6617	0.099	2.4, 8.5	33.6	20.8	1.11
	Apo6618	0.331	2.5, 8.6	33.6	20.5	1.49
	Apo6619	0.109	2.5, 8.6	33.4	20.7	1.11
	Apo6620	0.78	2.7, 8.7	33.8	20.3	1.46
	Apo6621	2.2	2.5, 8.7	34.3	20.9	1.65
	Apo6622	0.357	2.8, 8.6	33.9	20.8	1.28



**TABLE 2: Metal ion binding selectivity of Apo6619 ( $pK_{a1} = 2.5$ ,  $pK_{a2} = 8.6$ )**

Apo6619	Apo6619	Apo6619	Apo6619	Apo6619	Apo6619	Apo6619	Apo6619
log scale	Fe(III)	Al(III)	Cu(II)	Zn(II)	Mn(II)	Mg(II)	Ca(II)
$K_1$	12.5	9.3	8.9	6.3	5.1	4.1	3
$K_2$	11.6	9.5	7.7	5.8	4.3	3.2	2.1
$K_3$	9.5	8.2	-	-	-	-	-
$\beta_2$	-	-	16.6	12.1	9.4	7.3	5.1
$\beta_3$	33.6	27	-	-	-	-	-
PM	20.5	13.9	10	6.4	6.0	6.0	6.0

**TABLE 3: Effectiveness of Apo6619 and Apo6617 administered at a dose of 450  $\mu$ moles/kg in Promoting Fecal Iron Excretion in the Iron Overloaded Rats ( $n=6$ ). Values are expressed as  $\mu$ g/day/kg. Fecal excretion values three days after putative chelator administration are given. Values are expressed as mean  $\pm$  1SD.**

Test Article	Fecal excretion ( $\mu$ g/day/kg)
Baseline	3057 $\pm$ 184
Apo6619	4154 $\pm$ 1245
Apo6617	4411 $\pm$ 790

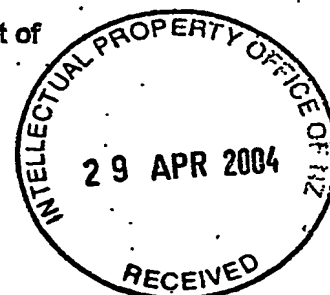
**TABLE 4: Effectiveness of Apo6619 and Apo6617 in Promoting Urinary and Fecal Iron Excretion in the Iron Overloaded Rats ( $n=6$ /group). Values are expressed as  $\mu$ g/day/kg. Fecal excretion values 3 days after chelator administration are given and compared to the baseline values determined 3 days prior to chelator administration. Values are expressed as mean  $\pm$  1SD.**

Iron excretion data expressed in $\mu$ g/day/kg ( $\pm$ SD)				
Compound	Urine (1 day post-chelator)		Feces (3 days) <sup>1</sup>	
	Apo6617	Apo6619	Apo6617	Apo6619
Dose Level				
0 (Baseline)	14 $\pm$ 4	11 $\pm$ 3	2300 $\pm$ 1003	2575 $\pm$ 871
28 $\mu$ mol/kg	24 $\pm$ 6*	14 $\pm$ 4	Not Measured	Not Measured
113 $\mu$ mol/kg	28 $\pm$ 8*	51 $\pm$ 15* $\tau$	2411 $\pm$ 335	3033 $\pm$ 1076
450 $\mu$ mol/kg	183 $\pm$ 20*	335 $\pm$ 76* $\tau$	3228 $\pm$ 437	3831 $\pm$ 790*

1- Assessment of 3-day fecal excretion was required to allow for transit of the iron through the gastrointestinal tract.

\* $p < 0.05$  versus baseline value in the same group

$\tau p < 0.05$  versus Apo6617 at the same dose





**TABLE 5:** Crystal data and structure refinement for Fe(Apo6617)<sub>3</sub>

Identification code	Fe(Apo6617) <sub>3</sub>	
Empirical formula	C <sub>33</sub> H <sub>42</sub> Fe N <sub>8</sub> O <sub>10.50</sub>	
Formula weight	746.58	
Temperature	150(1) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P -1	
Unit cell dimensions	a = 10.9760(4) Å	α = 94.283(2)°
	b = 11.3790(4) Å	β = 90.351(2)°
	c = 13.9952(5) Å	γ = 91.731(2)°
Volume	1742.18(11) Å <sup>3</sup>	
Z	2	
Density (calculated)	1.423 Mg/m <sup>3</sup>	
Absorption coefficient	0.500 mm <sup>-1</sup>	
F(000)	784	
Crystal size	0.30 x 0.14 x 0.04 mm <sup>3</sup>	
Theta range for data collection	2.62 to 25.00°	
Index ranges	-13 ≤ h ≤ 13, -13 ≤ k ≤ 13, -16 ≤ l ≤ 16	
Reflections collected	16641	
Independent reflections	6114 [R(int) = 0.0753]	
Completeness to theta = 25.00°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.966 and 0.892	
Refinement method	Full-matrix least-squares on F <sup>2</sup>	
Data / restraints / parameters	6114 / 2 / 462	
Goodness-of-fit on F <sup>2</sup>	1.034	
Final R indices [I > 2σ(I)]	R1 = 0.0566, wR2 = 0.1410	
R indices (all data)	R1 = 0.0830, wR2 = 0.1594	
Extinction coefficient	none	
Largest diff. peak and hole	0.609 and -0.539 e.Å <sup>-3</sup>	



TABLE 6. Bond lengths [Å] and angles [°] for Fe(Apo6617)<sub>3</sub>

Fe(1)-O(5)	1.985(2)	C(10)-C(11)	1.581(16)
Fe(1)-O(8)	2.010(2)	N(2*)-C(9*)	1.556(13)
Fe(1)-O(2)	2.016(2)	C(9*)-C(11*)	1.440(12)
Fe(1)-O(7)	2.020(3)	C(9*)-C(10*)	1.454(15)
Fe(1)-O(4)	2.025(2)	C(10*)-C(11*)	1.474(14)
Fe(1)-O(1)	2.048(2)	C(12)-C(13)	1.384(5)
O(1)-C(1)	1.294(4)	C(12)-C(17)	1.441(5)
O(2)-C(5)	1.327(4)	C(13)-C(14)	1.393(5)
O(4)-C(12)	1.299(4)	C(14)-C(18)	1.498(5)
O(5)-C(17)	1.321(4)	C(16)-C(17)	1.383(5)
O(6)-C(20)	1.233(4)	C(16)-C(20)	1.508(5)
O(7)-C(24)	1.297(4)	C(20)-N(4)	1.331(5)
O(8)-C(28)	1.329(4)	N(4)-C(21)	1.474(10)
O(9)-C(31)	1.218(4)	C(21)-C(23)	1.434(15)
N(1)-C(3)	1.351(5)	C(21)-C(22)	1.492(13)
N(1)-C(4)	1.397(5)	C(22)-C(23)	1.392(15)
N(1)-C(7)	1.487(5)	C(21*)-C(22*)	1.461(19)
N(3)-C(14)	1.355(5)	C(21*)-C(23*)	1.535(16)
N(3)-C(16)	1.390(5)	C(22*)-C(23*)	1.582(18)
N(3)-C(19)	1.483(5)	C(24)-C(25)	1.395(5)
N(5)-C(26)	1.361(5)	C(24)-C(28)	1.425(5)
N(5)-C(27)	1.387(4)	C(25)-C(26)	1.363(5)
N(5)-C(30)	1.474(5)	C(26)-C(29)	1.504(5)
C(1)-C(2)	1.393(5)	C(27)-C(28)	1.376(5)
C(1)-C(5)	1.434(5)	C(27)-C(31)	1.501(5)
C(2)-C(3)	1.383(6)	C(31)-N(6)	1.310(5)
C(3)-C(6)	1.495(5)	N(6)-C(32)	1.514(12)
C(4)-C(5)	1.384(5)	C(32)-C(34)	1.417(15)
C(4)-C(8)	1.493(6)	C(32)-C(33)	1.485(16)
C(8)-O(3*)	1.256(7)	C(33)-C(34)	1.459(15)
C(8)-O(3)	1.281(8)	C(32*)-C(33*)	1.433(16)
C(8)-N(2*)	1.325(8)	C(32*)-C(34*)	1.53(2)
C(8)-N(2)	1.378(8)	C(33*)-C(34*)	1.45(2)
N(2)-C(9)	1.608(12)	O(11)-O(11)#1	1.550(16)
C(9)-C(10)	1.447(16)	O(5)-Fe(1)-O(8)	88.36(9)
C(9)-C(11)	1.505(13)	O(5)-Fe(1)-O(2)	88.97(10)

TABLE 6. contd.

O(8)-Fe(1)-O(2)	95.35(10)	N(1)-C(4)-C(8)	119.3(3)
O(5)-Fe(1)-O(7)	166.27(10)	O(2)-C(5)-C(4)	124.3(3)
O(8)-Fe(1)-O(7)	80.41(10)	O(2)-C(5)-C(1)	115.1(3)
O(2)-Fe(1)-O(7)	99.86(11)	C(4)-C(5)-C(1)	120.6(3)
O(5)-Fe(1)-O(4)	81.11(10)	O(3 <sup>*</sup> )-C(8)-O(3)	26.2(4)
O(8)-Fe(1)-O(4)	101.01(10)	O(3 <sup>*</sup> )-C(8)-N(2 <sup>*</sup> )	118.8(5)
O(2)-Fe(1)-O(4)	160.54(10)	O(3)-C(8)-N(2 <sup>*</sup> )	117.9(6)
O(7)-Fe(1)-O(4)	93.23(10)	O(3 <sup>*</sup> )-C(8)-N(2)	117.3(6)
O(5)-Fe(1)-O(1)	103.01(10)	O(3)-C(8)-N(2)	127.8(6)
O(8)-Fe(1)-O(1)	167.38(10)	N(2 <sup>*</sup> )-C(8)-N(2)	22.3(4)
O(2)-Fe(1)-O(1)	79.61(10)	O(3 <sup>*</sup> )-C(8)-C(4)	125.0(4)
O(7)-Fe(1)-O(1)	89.01(10)	O(3)-C(8)-C(4)	119.1(5)
O(4)-Fe(1)-O(1)	86.34(10)	N(2 <sup>*</sup> )-C(8)-C(4)	116.2(4)
C(1)-O(1)-Fe(1)	113.1(2)	N(2)-C(8)-C(4)	112.9(4)
C(5)-O(2)-Fe(1)	114.4(2)	C(8)-N(2)-C(9)	106.3(6)
C(12)-O(4)-Fe(1)	112.7(2)	C(10)-C(9)-C(11)	64.7(7)
C(17)-O(5)-Fe(1)	113.2(2)	C(10)-C(9)-N(2)	103.8(9)
C(24)-O(7)-Fe(1)	113.6(2)	C(11)-C(9)-N(2)	113.2(8)
C(28)-O(8)-Fe(1)	112.7(2)	C(9)-C(10)-C(11)	59.4(7)
C(3)-N(1)-C(4)	121.3(3)	C(9)-C(11)-C(10)	55.8(7)
C(3)-N(1)-C(7)	117.7(3)	C(8)-N(2 <sup>*</sup> )-C(9 <sup>*</sup> )	119.2(7)
C(4)-N(1)-C(7)	120.8(3)	C(11 <sup>*</sup> )-C(9 <sup>*</sup> )-C(10 <sup>*</sup> )	61.2(7)
C(14)-N(3)-C(16)	121.3(3)	C(11 <sup>*</sup> )-C(9 <sup>*</sup> )-N(2 <sup>*</sup> )	114.6(8)
C(14)-N(3)-C(19)	118.6(3)	C(10 <sup>*</sup> )-C(9 <sup>*</sup> )-N(2 <sup>*</sup> )	100.4(9)
C(16)-N(3)-C(19)	120.1(3)	C(9 <sup>*</sup> )-C(10 <sup>*</sup> )-C(11 <sup>*</sup> )	58.9(7)
C(26)-N(5)-C(27)	120.5(3)	C(9 <sup>*</sup> )-C(11 <sup>*</sup> )-C(10 <sup>*</sup> )	59.8(7)
C(26)-N(5)-C(30)	120.7(3)	O(4)-C(12)-C(13)	125.6(3)
C(27)-N(5)-C(30)	118.7(3)	O(4)-C(12)-C(17)	116.3(3)
O(1)-C(1)-C(2)	124.9(4)	C(13)-C(12)-C(17)	118.9(3)
O(1)-C(1)-C(5)	117.8(3)	C(12)-C(13)-C(14)	120.9(3)
C(2)-C(1)-C(5)	117.3(3)	N(3)-C(14)-C(13)	120.2(3)
C(3)-C(2)-C(1)	121.3(4)	N(3)-C(14)-C(18)	119.4(3)
N(1)-C(3)-C(2)	120.4(3)	C(13)-C(14)-C(18)	120.5(4)
N(1)-C(3)-C(6)	119.4(4)	C(17)-C(16)-N(3)	119.7(3)
C(2)-C(3)-C(6)	120.2(4)	C(17)-C(16)-C(20)	120.0(3)
C(5)-C(4)-N(1)	119.0(3)	N(3)-C(16)-C(20)	120.3(3)
C(5)-C(4)-C(8)	121.6(3)	O(5)-C(17)-C(16)	123.9(3)

TABLE 6. contd.

O(5)-C(17)-C(12)	116.5(3)	C(28)-C(27)-C(31)	120.9(3)
C(16)-C(17)-C(12)	119.7(3)	N(5)-C(27)-C(31)	118.6(3)
O(6)-C(20)-N(4)	124.1(4)	O(8)-C(28)-C(27)	123.8(3)
O(6)-C(20)-C(16)	122.9(3)	O(8)-C(28)-C(24)	116.6(3)
N(4)-C(20)-C(16)	113.0(3)	C(27)-C(28)-C(24)	119.5(3)
C(20)-N(4)-C(21)	126.2(5)	O(9)-C(31)-N(6)	123.8(4)
C(23)-C(21)-N(4)	120.5(8)	O(9)-C(31)-C(27)	121.5(3)
C(23)-C(21)-C(22)	56.8(7)	N(6)-C(31)-C(27)	114.7(3)
N(4)-C(21)-C(22)	118.0(7)	C(31)-N(6)-C(32)	119.2(5)
C(23)-C(22)-C(21)	59.5(7)	C(34)-C(32)-C(33)	60.3(8)
C(22)-C(23)-C(21)	63.7(8)	C(34)-C(32)-N(6)	126.8(9)
C(22*)-C(21*)-C(23*)	63.7(8)	C(33)-C(32)-N(6)	118.8(8)
C(21*)-C(22*)-C(23*)	60.4(8)	C(34)-C(33)-C(32)	57.5(7)
C(21*)-C(23*)-C(22*)	55.9(8)	C(32)-C(34)-C(33)	62.2(7)
O(7)-C(24)-C(25)	125.8(3)	C(33*)-C(32*)-C(34*)	58.7(9)
O(7)-C(24)-C(28)	116.6(3)	C(32*)-C(33*)-C(34*)	64.0(9)
C(25)-C(24)-C(28)	117.5(3)	C(33*)-C(34*)-C(32*)	57.3(8)
C(26)-C(25)-C(24)	121.9(4)		
N(5)-C(26)-C(25)	120.1(3)		
N(5)-C(26)-C(29)	118.4(3)		
C(25)-C(26)-C(29)	121.5(4)		
C(28)-C(27)-N(5)	120.4(3)		

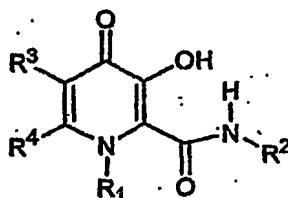
Symmetry transformations used to  
generate equivalent atoms:

#1 -x, -y+1, -z



**CLAIMS:**

1. A 3-hydroxypyridin-4-one compound of formula I:



wherein:

R<sup>1</sup> is X with the proviso that R<sup>2</sup> is Y;

or

R<sup>1</sup> is T with the proviso that R<sup>2</sup> is W;

X is C<sub>3</sub>-C<sub>6</sub> cycloalkyl;

Y is selected from the group C<sub>3</sub>-C<sub>6</sub> cycloalkyl; C<sub>1</sub> to C<sub>6</sub> alkyl;

T is selected from the group C<sub>1</sub> to C<sub>6</sub> alkyl;

W is C<sub>3</sub>-C<sub>6</sub> cycloalkyl;

R<sup>3</sup> is selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl;

R<sup>4</sup> is selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl;

and its pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein R<sup>1</sup> is X with the proviso that R<sup>2</sup> is Y.

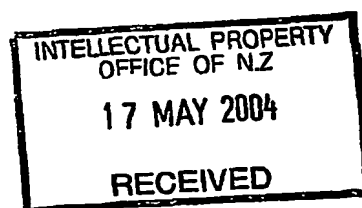
3. A compound of claim 2 wherein X is C<sub>3</sub> to C<sub>6</sub> cycloalkyl, and Y is C<sub>1</sub> to C<sub>6</sub> alkyl.

4. A compound of claim 3 wherein X is cyclopropyl, Y is methyl, R<sup>3</sup> is hydrogen and R<sup>4</sup> is methyl.

5. A compound according to claim 1 wherein R<sup>1</sup> is T with the proviso that R<sup>2</sup> is W.



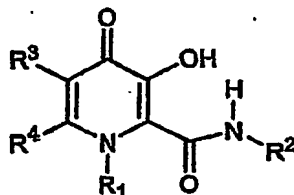
6. A compound of claim 5 wherein T is methyl, W is cyclopropyl, R<sup>3</sup> is hydrogen and R<sup>4</sup> is methyl.
- 5 7. A compound of claim 2 wherein X is C<sub>3</sub> to C<sub>6</sub> cycloalkyl, and Y is C<sub>3</sub> to C<sub>6</sub> cycloalkyl.
8. A compound of claim 7 wherein X is cyclopropyl, Y is cyclopropyl, R<sup>3</sup> is hydrogen and R<sup>4</sup> is methyl.
- 10 9. A compound of claim 3, the compound is 1-cyclopropyl-3-hydroxy-6-methyl-4-oxo-1, 4-dihydro-pyridine-2-carboxylic acid methylamide.
10. A compound of claim 6, the compound is 3-hydroxy-1, 6-dimethyl-4-oxo-1, 4-dihydro-pyridine-2-carboxylic acid cyclopropylamide.
- 15 11. A pharmaceutical composition comprising a compound according to claim 1 and a physiologically acceptable carrier.
12. A pharmaceutical composition according to claim 1, which is adopted for oral administration.
- 20 13. The use of a compound as defined under claim 1 in the manufacture of medicament for use in the treatment of a medical condition related to a toxic concentration of iron.



**ABSTRACT**

10     The present invention involves cycloalkyl derivatives of 3-hydroxy-4-pyridinone, which are useful for the chelation of metal ions such as iron. Its preparation and use are described. In particular, the invention concerns the removal of iron in chemical and biological systems comprising chelating agents having the formula

15



wherein R<sup>1</sup> is X with the proviso that R<sup>2</sup> is Y; or R<sup>1</sup> is T with the proviso that R<sup>2</sup> is W. X is C<sub>3</sub>-C<sub>6</sub> cycloalkyl; Y is C<sub>3</sub>-C<sub>6</sub> cycloalkyl; C<sub>1</sub> to C<sub>6</sub> alkyl. T is C<sub>1</sub> to C<sub>6</sub> alkyl. W is C<sub>3</sub>-C<sub>6</sub> cycloalkyl. R<sup>3</sup> is selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl. R<sup>4</sup> is selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>4</sub> alkyl. A pharmaceutical composition of such compounds is useful in the removal of excess body iron from patients with iron overload diseases.

20



1/4

Figure 1: Job's Plot for Apo6622  
 $[\text{Fe}^{3+}]_{\text{total}} + [\text{Apo6622}]_{\text{total}} = \text{Constant} = 8 \times 10^{-4} \text{ M}$   
Solvent: 0.1 M MOPS pH=7.4 Buffer

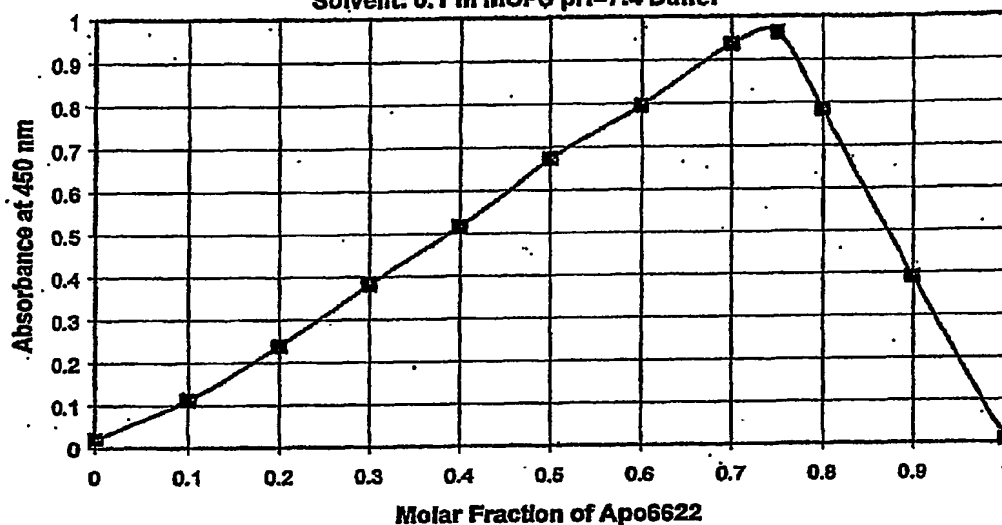


Figure 2: Job's Plot for Apo6617  
 $[\text{Fe}^{3+}]_{\text{total}} + [\text{Apo 6617}]_{\text{total}} = 8 \times 10^{-4} \text{ M}$   
Solvent: 0.1 M MOPS Buffer pH 7.4

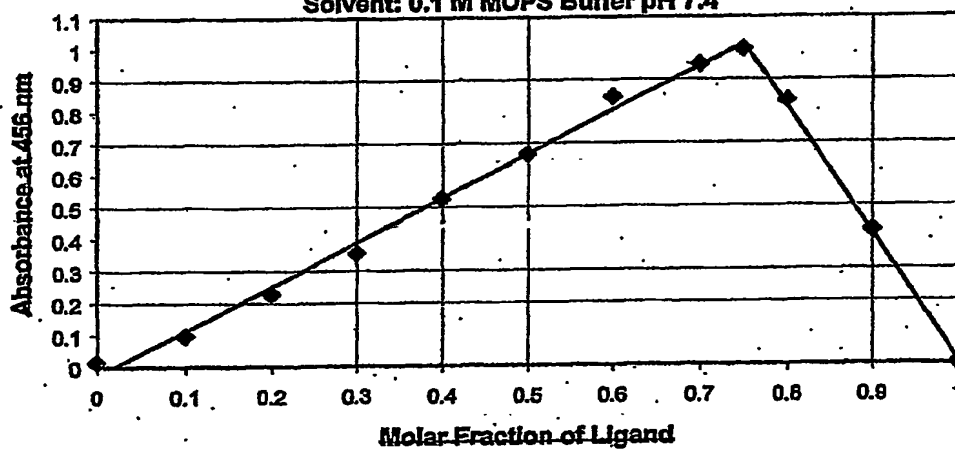
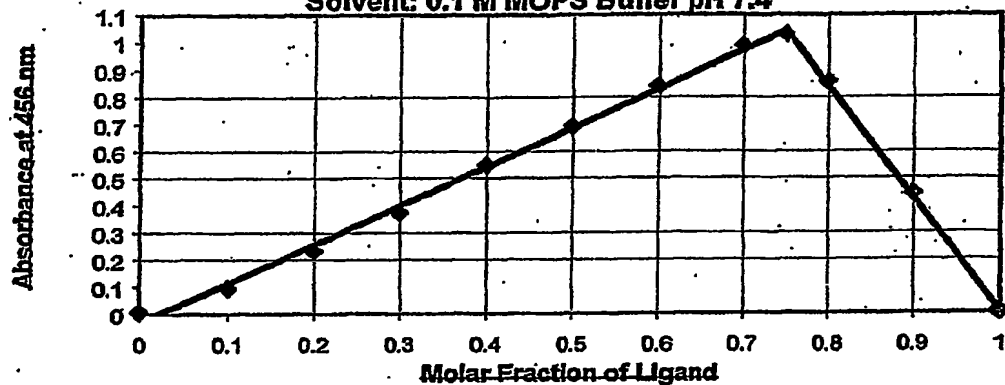




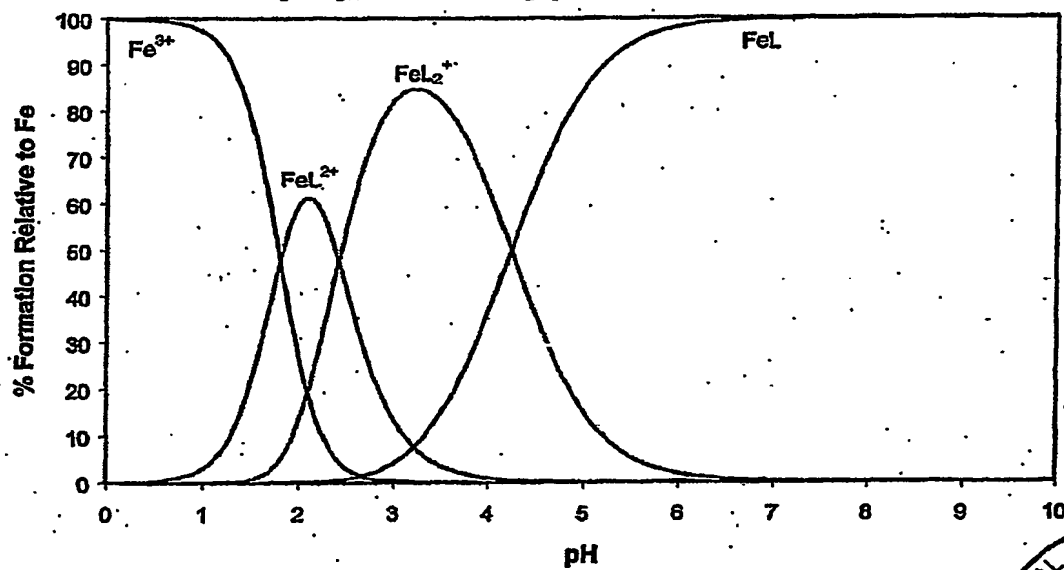
Figure 3: Job's Plot for Apo 6619

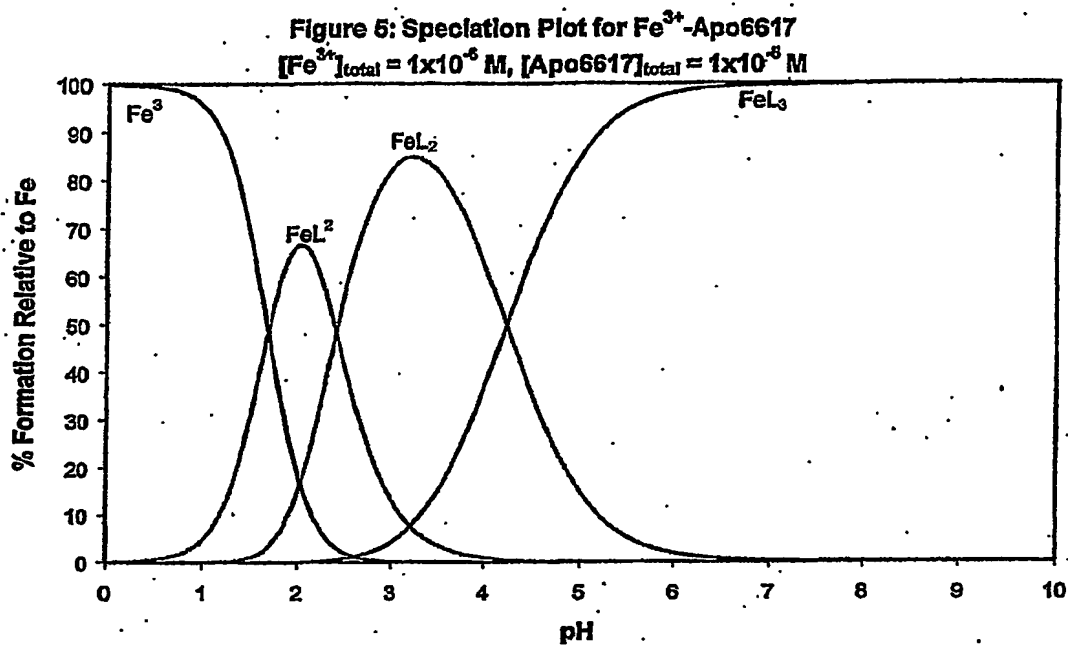
$$[\text{Fe}^{3+}]_{\text{total}} + [\text{Apo 6619}]_{\text{total}} = 8 \times 10^{-4}$$

Solvent: 0.1 M MOPS Buffer pH 7.4

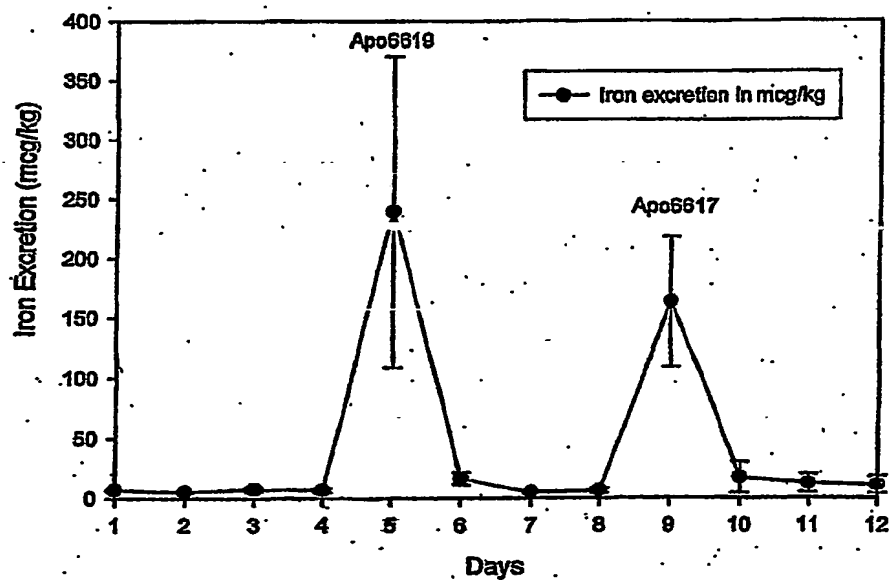
Figure 4: Speciation Plot for Fe<sup>3+</sup>-Apo6619

$$[\text{Fe}^{3+}]_{\text{total}} = 1 \times 10^{-6} \text{ M}, [\text{Apo6619}]_{\text{total}} = 1 \times 10^{-5} \text{ M}$$

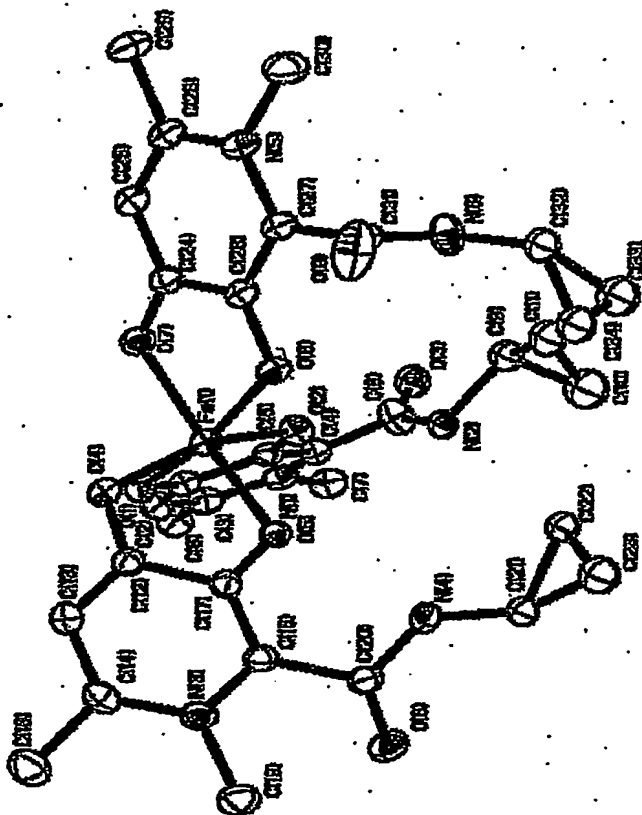


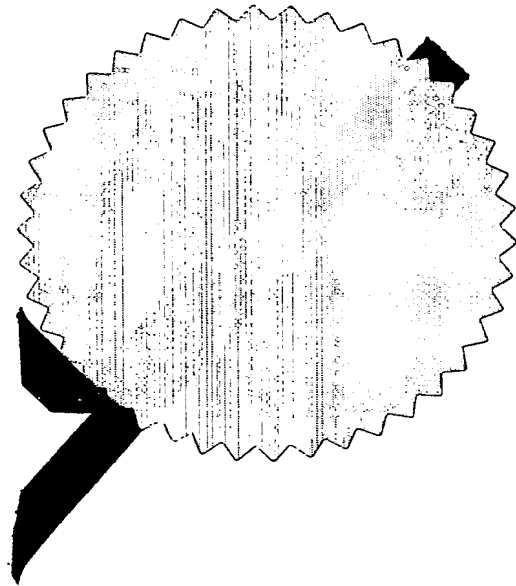


**Figure 6**  
Effectiveness of Apo6619 and Apo6617 in Promoting Urinary Iron Excretion



**Figure 7.**  
The crystal structure of  $\text{Fe}(\text{Apo6617})_3$ .





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